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Beurteilung der Therapietreue in der antihypertensiven Therapie basierend auf Therapeutischem Drug Monitoring (TDM)

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“Give the ones you love

wings to fly,

roots to come back

and **reasons** to stay.”

– Dalay Lama XIV

Für die Menschen, die mir **Flügel**,

Wurzeln und **Gründe** geben.

INHALTSVERZEICHNIS

1 Zusammenfassung

Hypertonie stellt in der westlichen Welt die Haupttodesursache dar, obwohl sie im Hinblick auf die pharmakologischen Therapieoptionen gut behandelbar ist. Hauptursächlich ist hierfür eine, vor allem durch eine Non-Adhärenz (u.a. bedingt durch den asymptomatischen Charakter) und in selteneren Fällen eine aufgrund einer therapieresistente Hypertonie (TRH) verursachte, unzureichende Blutdruckkontrolle. Eine Möglichkeit zur Überprüfung der Therapietreue in der antihypertensiven Therapie ist der qualitative Nachweis der Arzneistoffe im Blut oder Urin. Unklar ist, inwiefern die Substanzen in einer biologischen Probe innerhalb des Dosierungsintervalls oder darüber hinaus nachweisbar sind und es zu einer Falschbeurteilung kommen kann.

Daher wurde eine quantitative chromatographisch-tandem-massenspektrometrische Methode für das Therapeutische Drug Monitoring von blutdrucksenkenden Arzneistoffen entwickelt und analytisch vollständig validiert. Bei 38 Patienten mit überwachter Medikamenteneinnahme wurde die Aussagekraft der Methode hinsichtlich der Bestätigung einer Adhärenz zunächst mittels zweier Wirkstoffkonzentrationen im Serum (Tal- und Spitzenspiegel) überprüft. Zur Bewertung der Konzentrationen wurden zwei Konzepte evaluiert. Einerseits wurde die untere Grenze des therapeutischen Referenzbereiches (TRR, Literaturdaten) und andererseits die mittels Rechenmodell (Daten pharmakokinetischer Studien) individuell ermittelte, dosisbezogene Arzneimittelkonzentration (DRC) evaluiert. In einem zweiten Studienansatz wurde diese neue quantitative Methode an einem Kollektiv von 36 ambulanten Patienten (ohne überwachte Medikamenteneinnahme) angewendet und zur Überprüfung der Aussagekraft mit Ergebnissen des etablierten Urinscreenings verglichen.

Die gemessenen Wirkstoffkonzentrationen von Atenolol (64 bis 564 ng/ml), Bisoprolol (2,5 bis 53 ng/ml), Metoprolol (5,8 bis 110 ng/ml), Nebivolol (0,32 bis 3,4 ng/ml), Hydrochlorothiazid (15 bis 606 ng/ml), Furosemid (22 ng/ml), Torasemid (17 bis 1829 ng/ml), Canrenon (25 bis 221 ng/ml), Amlodipin (2,4 bis 35 ng/ml), Lercanidipin (0,24 bis 21 ng/ml), Candesartan (6,0 bis 268 ng/ml), Telmisartan (22 bis 375 ng/ml) und Valsartan (115 bis 7962 ng/ml) haben gezeigt, dass die quantitative Analyse von Antihypertensiva in Serumproben und deren

Auswertung auf Basis der individuell berechneten unteren DRC in der Beurteilung einer Adhärenz vielversprechend ist.

Die Auswertung auf Basis der unteren Grenze des TRR signalisierte bei den stationären Patienten (überwachte Einnahme) innerhalb der Substanzklasse der Diuretika (ohne Torasemid) bei 16,7 %, der β-Blocker bei 29,4 %, der Calciumkanal-Blocker bei 14,8 % und der AT1-Antagonisten bei 25 % fälschlicherweise eine Non-Adhärenz.

Die rein qualitative Urinanalyse zeigte im Falle der β-Blocker Atenolol, Bisoprolol und des Diuretikums HCT aufgrund einer hohen Bioverfügbarkeit, einer langen Halbwertszeit oder einer überwiegend renalen Ausscheidung der Muttersubstanz ein Nachweisfenster über das Dosierungsintervall hinaus, was bedingt, dass einige Patienten fälschlicherweise als adhärent gewertet wurden. Ein anderes Problem zeigte sich bei einigen Patienten, die mit dem AT1-Antagonist Candesartan oder dem Calciumkanal-Blocker Lercanidipin behandelt wurden und die als non-adhärent eingestuft wurden. Eine geringe Bioverfügbarkeit, eine hohe Metabolisierungsrate oder geringe renale Ausscheidung der unveränderten Arzneistoffe lies auf eine mangelhafte Nachweisbarkeit innerhalb des Dosierungsintervalls und damit auf eine eingeschränkte Beurteilbarkeit schließen.

Aus den Untersuchungen ergibt sich, dass es bei Anwendung qualitativer Nachweismethoden aufgrund besonderer Pharmakokinetiken einzelner antihypertensiver Wirkstoffe zur Fehleinschätzung der Adhärenz kommen kann. Die neu entwickelte Methodik in Form einer quantitativen Serumanalyse ist unter Verwendung patientenindividueller Bewertungskriterien bei dieser Fragestellung überlegen.

2 Abstract

Hypertension is the main cause of death in the western world, despite the wide range of pharmacological treatment options. This is predominantly caused by insufficient blood pressure control, mainly due to non-adherence (inter alia due to its asymptomatic character) and more rarely to therapy-resistant hypertension (TRH). One possibility for adherence assessment in antihypertensive therapy is the qualitative detection of the drugs in blood or urine. What remains to be clarified is the extent to which the drugs are detectable in a biological sample within or beyond the dosage interval and whether this leads to a misjudgement.

Therefore, a quantitative chromatographic tandem mass spectrometric method for therapeutic drug monitoring of antihypertensive drugs was developed and fully validated analytically. In 38 patients with monitored drug intake, the validity of the method was first evaluated based on two drug concentrations in the serum (trough and peak levels) to confirm adherence. Two approaches were evaluated to assess concentrations. On the one hand, the lower limit of the therapeutic reference range (TRR, literature data) and on the other hand the dose-related drug concentration (DRC) determined individually using a mathematical model (data from pharmacokinetic studies) were evaluated. In a second study approach, this new quantitative method was applied to a collective of 36 outpatients (without monitored drug intake) and compared with the results of the established qualitative urinalysis to verify the validity of the results.

The measured concentrations of atenolol (64 to 564 ng/ml), bisoprolol (2.5 to 53 ng/ml), metoprolol (5.8 to 110 ng/ml), nebivolol (0.32 to 3,4 ng/ml), hydrochlorothiazide (15 to 606 ng/ml), furosemide (22 ng/ml), torasemide (17 to 1829 ng/ml), canrenone (25 to 221 ng/ml), amlodipine (2.4 to 35 ng/ml), lercanidipine (0.24 to 21 ng/ml), candesartan (6.0 to 268 ng/ml), telmisartan (22 to 375 ng/ml) and valsartan (115 to 7962 ng/ml) proved that the quantitative analysis of antihypertensive drugs in serum samples and the evaluation based on the individually calculated lower DRC is promising in the assessment of adherence.

Evaluation based on the lower limit of TRR falsely indicated non-adherence in the inpatients (monitored drug intake) for 16.7% of diuretics (except torasemide),

29.4% of β blockers, 14.8% of calcium-channel blockers and 25% of angiotensin receptor blockers.

In case of the β-blockers atenolol, bisoprolol and the diuretic HCT, the qualitative urinalysis showed a detectability beyond the dosage interval due to high bioavailability, a long half-life or a predominantly renal excretion of the parent substance, which led some patients to be falsely considered adherent. Another problem was observed in some patients treated with the angiotensin receptor blocker candesartan or the calcium-channel blocker lercanidipine being classified as non-adherent. A low bioavailability, a high metabolisation rate or a low renal excretion of the unchanged drugs indicated poor detectability within the dosage interval and thus limited assessability.

The study results show that the use of qualitative detection methods may lead to misinterpretation of adherence due to the specific pharmacokinetics of an antihypertensive drug. The newly developed methodology based on quantitative serum analysis using patient-specific evaluation criteria is superior.

3 Übergreifende Zusammenfassung

3.1 Einleitung

Die arterielle Hypertonie gilt weltweit nicht nur als Hauptrisikofaktor für die Entwicklung kardiovaskulärer Erkrankungen, sondern stellt zugleich auch die häufigste Todesursache in der westlichen Welt dar [1]. Sie betrifft mittlerweile mehr als ein Drittel der erwachsenen Bevölkerung [2] (30,9 % Frauen, 32,8 % Männer) mit einem Anstieg der Prävalenz auf zwei Drittel der Population der ≥ 65-Jährigen [3]. Trotz der Vielzahl an pharmakologischen Therapieoptionen (Substanzklassen: ACE-Hemmer, AT1-Antagonisten, β-Blocker, Calciumkanal-Blocker und Diuretika) ist die Blutdruckeinstellung häufig unzureichend. Diesbezüglich zählt eine fehlende Therapietreue zu den Hauptgründen für das Versagen der Bluthochdrucktherapie [4]. Die Therapietreue, insbesondere die Adhärenz, beschreibt den Grad, in welchem sich der Patient hinsichtlich seiner Medikamenteneinnahme an das mit dem Arzt vereinbarte und gegenseitig akzeptierte Therapieregime hält [5]. Eine fehlende Therapietreue (Non-Adhärenz) wird nicht nur für eine erhöhte Belastung des Gesundheitssystems (häufige Arztbesuche, Krankenhausaufenthalte), sondern auch für den Wirkverlust einer Therapie verantwortlich gemacht [6]. Um die Patientensicherheit zu erhöhen und die Gesundheitskosten zu senken, müssen sich Patienten an ihr Therapieschema halten. Allerdings ist eine fehlende Therapietreue in der Bluthochdrucktherapie keine Seltenheit. Dies spiegelt sich in Adhärenz-Raten zwischen 50 % und 70 % wieder [4]. Ursächlich für diese Schwankungsbreite sind einerseits die unterschiedlichen Definitionen von Adhärenz sowie deren Differenzierung (Cut-off: ≥ 80 % oder 100 %; Klassifizierung: niedrig, mittel, hoch) [7,8], andererseits variiert die Rate aber auch in Abhängigkeit von der angewandten Messmethode. Während in einer auf Nachfüllstatistiken beruhende Meta-Analyse für die Substanzklassen der Diuretika und β-Blocker die niedrigste Therapietreue (< 51 %) ermittelt wurde [9], beschreiben Studien, welche ihre Daten mittels qualitativer Urin- oder Blutanalysen ermittelt haben für diese beiden Klassen die höchsten Adhärenz-Raten (> 91 %) [10,11].

Ein weiterer Grund für das Versagen der Bluthochdrucktherapie kann das Vorliegen einer therapieresistenten Hypertonie (TRH; Prävalenz: 10 – 20 %) [12,13] sein. In diesem Fall ist der Blutdruck trotz der maximal tolerierbaren Dosisgabe

von einem Diuretikum und mindestens zwei weiteren Antihypertensiva verschiedener Substanzklassen nicht kontrollierbar [14,15]. Um die Adhärenz eines Patienten zu ermitteln und u.a. eine TRH auszuschließen, stehen verschiedene indirekte und direkte Methoden zur Verfügung. Als indirekte Methoden finden u.a. Patiententagebücher, Interviews, Fragebögen, Statistiken zu Verschreibungshäufigkeiten, Tablettenzählen („Pill Count“) und elektronische Beobachtungssysteme wie MEMS® („Medication Event Monitoring System“) Anwendung [16]. Zu den direkten Messmethoden zählen z.B. die direktüberwachte Medikamenteneinnahme (*engl.* directly observed therapy, DOT) und das Therapeutische Arzneimittel Monitoring (*engl.* therapeutic drug monitoring, TDM). Alle genannten Methoden haben ihr Für und Wider. Allerdings empfiehlt es sich, aufgrund der Möglichkeit des Patienten indirekte Methoden zu manipulieren, den direkten Methoden den Vorzug zu geben [17]. Als direkte Messmethode hat sich die Identifizierung eines breiten Spektrums an blutdrucksenkenden Medikamenten im Serum, Plasma und Urin über die letzten Jahre etabliert [16,11,10], wobei diese Messungen nur von wenigen Laboren angeboten werden. Dennoch kann auch ein rein qualitativer Nachweis aus pharmakokinetischen Gründen (z.B. Bioverfügbarkeit, Metabolisierung) zu einer Fehleinschätzung der Adhärenz führen. Dies betrifft sowohl Arzneistoffe, die nicht über das komplette Dosierungsintervall als auch solche die über dieses Intervall hinaus nachweisbar sind. Dementsprechend ist eine Quantifizierung im Blut erforderlich. Kürzlich wurde eine solche Methode zur Wirkstoffkonzentrationsbestimmung von antihypertensiven Arzneistoffen im Blut mittels flüssigchromatographisch-tandem-massenspektrometrischem Verfahren (LC-MS/MS) publiziert [18]. Die Kopplungstechnik bietet die Möglichkeit eine Vielzahl von Substanzen auch bei bereits sehr geringen Analyt-Konzentrationen extrem empfindlich nachzuweisen. Der publizierten Methode mangelt es dennoch an Grenzwerten, die eine Adhärenz von einer Non-Adhärenz unterscheiden. Im Therapeutischen Drug Monitoring (TDM) dient als Bezugssystem zur Einschätzung der Wirkstoffkonzentration der therapeutische Referenzbereich (*engl*. therapeutic reference range, TRR), welcher dazu dient die Konzentration eines Wirkstoffes hinsichtlich seiner therapeutischen Effektivität zu werten. Hierbei zeigt das Überschreiten der Untergrenze eine Wirkung (minimal therapeutische Konzentration) und das Überschreiten der Obergrenze (minimal toxische Konzentration) das Auftreten von unerwünschten

Arzneimittelwirkungen (UAWs) an. Ein weiterer Parameter ist der dosisbezogene Referenzbereich, welcher den Konzentrationsbereich beschreibt, der aufgrund der applizierten Dosis im Blut zu erwarten ist [19].

Auf Grundlage dessen zielt die vorliegende Arbeit darauf ab, eine quantitative LC-MS/MS-Methode zu entwickeln und eine Bewertungsgrundlage zu schaffen, die dazu dient, Adhärenz bei Bluthochdruckpatienten – verglichen mit aktuell angewandten Screening-Methoden – präziser zu diagnostizieren.

3.2 Darstellung der Publikationen und weiterführender Forschungsaspekte

Im Folgenden sind die Publikationen sowie weiterführende Forschungsaspekte dargestellt, die im Rahmen der Entwicklung einer LC-MS/MS-basierten quantitativen Methode und deren Vergleich mit einem etablierten qualitativen Urinscreening zur Adhärenz-Bewertung entstanden sind. Die Auswahl der Substanzen konzentriert sich hierbei auf die in Deutschland überwiegend verschriebenen Medikamente der Substanzklassen der Diuretika, β-Blocker, AT1-Antagonisten und Calciumkanal-Blocker. Die Daten zur Verschreibungshäufigkeit basieren hierbei auf dem jährlich erscheinenden Arzneiverordnungsreport (AVR) des Wissenschaftlichen Instituts der Allgemeinen Ortskrankenkassen (WIdO).

3.2.1 Publikation1: Evaluation of the dose-related concentration approach in therapeutic drug monitoring of diuretics and β-blockers – drug classes with low adherence in antihypertensive therapy

Der Nachweis von blutdrucksenkenden Medikamenten in biologischen Proben ist ein wichtiges Verfahren um die Therapietreue bei Bluthochdruckpatienten zu beurteilen. Allerdings bergen Methoden basierend auf qualitativen Ergebnissen die Gefahr einer Fehleinschätzung der Adhärenz. Dies kann z.B. Arzneistoffe betreffen, die ein langes Nachweisfenster in der biologischen Probe aufweisen. Aus diesem Grund beschäftigt sich die Originalarbeit mit der Entwicklung einer Methode, die für das Therapeutische Arzneimittel Monitoring (TDM) bei Patienten unter β-Blocker- oder Diuretika-Therapie zur Adhärenz-Bewertung eingesetzt

werden kann. Der Fokus auf diesen Substanzklassen liegt in den, laut einer Meta-analyse, niedrigen Adhärenz-Raten begründet.

Zunächst wurde zur Quantifizierung der β-Blocker und Diuretika ein LC-MS/MS basiertes Verfahren entwickelt und validiert. Die Methode wurde anschließend zur Wirkstoffkonzentrationsmessung bei 20 Patienten angewandt, die einer beobachteten Einnahme unterlagen. Zur Grenzwertfindung wurden Tal- und Spitzenspiegel der Patienten gemessen und die Aussagekraft zweier Konzepte hinsichtlich der Adhärenz-Bewertung getestet. Hierzu dienten einerseits Literaturdaten zu therapeutischen Konzentrationsbereichen (therapeutischer Referenzbereich, TRR), andererseits wurden mittels Daten aus pharmakokinetischen Studien individuelle dosisbezogene Arzneimittelkonzentrationen (*engl.* dose-related concentration, DRC) berechnet. Hierzu wurde die errechnete Konzentration um eine Standardabweichung (SD) nach unten korrigiert um individuelle pharmakokinetische Unterschiede hinreichend zu berücksichtigen (DRC-SD). Dieser Wert wurde dann zur Konformitätsbewertung herangezogen. Beide Konzepte wurden anschließend dahingehend überprüft, inwieweit sie als zuverlässiger Cut-off für die Adhärenz-Bewertung dienen.

In der Studie hat sich gezeigt, dass die gemessenen Konzentrationen in 24,1 % der Probandenproben unterhalb des TRR lagen. Dies betraf mit 17,2 % hauptsächlich die Substanzklasse der β-Blocker und mit einem geringeren Anteil von 6,9 % die Klasse der Diuretika. Im Gegensatz zum TRR lagen alle gemessenen Konzentrationen (Tal- und Spitzenspiegel) von Bisoprolol (3,3 bis 53 ng/ml), Metoprolol (5,8 bis 110 ng/ml), Nebivolol (0,36 und 1,0 ng/ml), Hydrochlorothiazid (15 bis 606 ng/ml), Torasemid (17 bis 1829 ng/ml) und Canrenon (25 bis 100 ng/ml) oberhalb der individuell berechneten unteren dosisbezogenen Konzentration und in Literatur gestützten Bereichen.

Nach den Ergebnissen der Studie scheint die Konzentrationsbestimmung gekoppelt an die individuelle Berechnung der unteren dosisbezogenen Konzentration dem therapeutischen Referenzbereich hinsichtlich der Adhärenz-Bewertung überlegen. In der Studie hat es sich als ein vielversprechendes Verfahren präsentiert, um Adhärenz zuverlässig zu beurteilen. Diese Methode könnte möglicherweise eine fehlende Therapietreue oder andere Ursachen für eine unzureichende Therapie verlässlicher erkennen als qualitative Methoden.

3.2.2 Publikation 2: Assessment of adherence to diuretics and β-blockers by serum drug monitoring in comparison to urine analysis

Screenings zur Identifizierung von blutdrucksenkenden Medikamenten haben im letzten Jahrzehnt zunehmend an Bedeutung gewonnen und gelten als nützliches Verfahren zur Adhärenz-Bewertung. Ein mögliches Problem bei rein qualitativen Nachweismethoden könnten Arzneistoffe sein, die einer hohen Metabolisierungsrate unterliegen oder kaum renal ausgeschieden werden sowie solche, die aufgrund ihrer langen Halbwertszeit ein verlängertes Nachweisfenster aufweisen. Dies könnte zu einer Fehleinschätzung bezüglich der Therapietreue führen. Aus diesem Grund beschäftigt sich die vorliegende Studie mit dem Vergleich zweier Methoden, die zur Beurteilung der Adhärenz in der Bluthochdrucktherapie dienen. Zum einen handelt es sich hierbei um das unter 3.2.1 entwickelte Verfahren und zum anderen um eine qualitative Urinscreening-Methode, welche bereits für die Beurteilung der Adhärenz in der Bluthochdrucktherapie Anwendung findet.

Um die Zuverlässigkeit der beiden Methoden zu überprüfen wurde 26 ambulanten Patienten, welche keiner überwachten Medikamenteneinnahme unterlagen, zum gleichen Zeitpunkt Urin und Blut entnommen. Die Proben wurden anschließend mittels LC-MS/MS untersucht. Bei den Urinproben galt die Adhärenz als belegt, wenn die verschriebenen Medikamente im Urin sicher nachweisbar waren, d.h. oberhalb der Nachweisgrenze (*engl.* limit of detection, LOD) lagen. In den Blutproben hingegen musste die Wirkstoffkonzentration eines Patienten für den jeweiligen Arzneistoff über der individuell berechneten unteren dosisbezogenen Konzentration (DRC-SD) und im Rahmen einer weiteren Betrachtung über der unteren Grenze des in der Literatur beschriebenen TRR liegen.

Insgesamt wurden mittels Urinanalyse 88,5 % (n=23), durch die Serumanalyse mit dem DRC-SD als Cut-off-Wert 80,8 % (n=21) und anhand des TRR 50,0 % (n=13) der Patienten als adhärent klassifiziert. Unterschiede zeigten sich beim Vergleich der Ergebnisse zwischen der Urinanalytik und der Serumanalyse basierend auf den individuell errechneten Grenzwerten in 15,0 % (n=6) der Verschreibungen. Dies betraf mit 12,5 % hauptsächlich die Gruppe der β-Blocker

mit Atenolol, Bisoprolol und Nebivolol, was in den pharmakokinetischen Eigenschaften der Substanzen begründet liegt. Während ein hoher Anteil an Atenolol und Bisoprolol renal unverändert ausgeschieden wird, unterliegt Nebivolol einer starken Verstoffwechselung was sich in einer renalen Wirkstoffausscheidung < 0,5 % widerspiegelt.

Diese Studie bestätigt die Annahme, dass die Adhärenz-Beurteilung auf Grundlage der entwickelten Serummethode bezogen auf die DRC-SD zuverlässiger ist als die qualitative Urinanalytik.

3.2.3 Weiterführende Forschungsaspekte: Beurteilung der Adhärenz bezüglich AT1-Antagonisten und Calciumkanal-Blockern mittels Serum- und Urinmonitoring

Die zunehmende Zahl an Bluthochdruck-Neuerkrankungen sorgt für einen Anstieg der Verschreibungsraten. Allerdings zeigt sich seit 2014 eine Trendwende bezüglich der Verschreibungen in Deutschland. Während ACE-Hemmer, Betablocker und Diuretika in der Verschreibungshäufigkeit rückläufig waren, stiegen die Zahlen der AT1-Antagonisten und Calciumkanal-Blocker an. Ursächlich hierfür könnte sein, dass Bluthochdruck-Patienten zunehmend den Symptomenkomplex des metabolischen Syndroms aufweisen. In diesem Patientenkollektiv, welches mittlerweile ein Drittel der Bluthochdruckpatienten darstellt, wird von der Therapie mittels Diuretika und Betablocker abgeraten, da diese ein diabetogenes Potential bergen.

Ziel der vorliegenden Studie war es, eine auf einem TDM basierende Methode bei stationären Patienten zu validieren, welche die beiden in der Verschreibungshäufigkeit steigenden Substanzkassen erfasst und mit einem etablierten Urinscreening zur Adhärenz-Beurteilung bei ambulanten Patienten zu vergleichen. Hierzu wurde das bereits entwickelte Verfahren (unter 3.2.1) zur Quantifizierung von Diuretika und β-Blockern um die zwei Substanzklassen der Calciumkanal-Blocker und AT1-Antagonisten erweitert (*Tabelle 1, Tabelle 2*). Anschließend wurde die Methode bei 32 stationären Patienten angewendet, die einer überwachten Medikamenteneinnahme unterlagen, um die individuell errechneten unteren dosisbezogenen Konzentrationen (DRC-SD) sowie die untere Grenze

des TRR als möglichen Cut-off-Wert zu überprüfen und festzulegen (Patientenkollektiv und Methodik wie in 3.2.1). Darüber hinaus wurden von 22 ambulanten, bei der Medikamenteneinnahme unbeaufsichtigten Patienten zeitgleich Urin- und Blutproben entnommen und untersucht (Patientenkollektiv und Methodik wie in 3.2.2). Dies ermöglicht den Vergleich der quantitativen Serum- mit der qualitativen Urin-Methode hinsichtlich der Adhärenz-Beurteilung.

Ambulante Patienten:

Die gemessenen Konzentrationen (Tal- und Spitzenspiegel) der stationär-überwachten Patienten lagen mit Amlodipin (2,4 bis 35 ng/ml), Lercanidipin (0,24 bis 21 ng/ml), Candesartan (6,0 bis 268 ng/ml), Telmisartan (22 bis 101 ng/ml) und Valsartan (115 bis 7962 ng/ml) oberhalb der individuell berechneten unteren dosisbezogenen Konzentration und innerhalb der in der Literatur beschriebenen Bereiche. Bezogen auf den TRR lagen die gemessenen Konzentrationen in 82,1 % der Probandenproben oberhalb der unteren Grenze des TRR.

Stationäre Patienten:

Bei dem Vergleich der Ergebnisse der Urin- und Serummethode, einmal basierend auf der DRC-SD und zweitens auf dem TRR, suggerierte die Urinanalytik bei 77,3 % (n=17) und bei der Serummethodik mittels DRC-SD 86,4 % (n=19) sowie mittels TRR bei 68,2 % (n=15) der ambulanten Patienten ein adhärentes Therapieverhalten. Unterschiede zwischen der Urin- und Serumanalytik basierend auf der unteren dosisbezogenen Konzentration zeigten sich beim Vergleich der Ergebnisse in 9,1 % (n=2) der Patienten. Das betraf einen von 3 Patienten mit Candesartan-Verschreibung und einen von 5 Patienten, welche Lercanidipin erhielten. Beide Patienten wurden anhand der Serummethode als adhärent und mittels Urinanalyse als non-adhärent klassifiziert. Ursächlich für die fehlende Nachweisbarkeit im Urin kann die starke Metabolisierung von Lercanidipin und im Falle von Candesartan die geringe Bioverfügbarkeit gekoppelt an eine hohe Stoffwechselrate sein.

Auch für die Stoffgruppen der AT1-Antagonisten und Calicumkanal-Blocker konnte gezeigt werden, dass die Adhärenz-Bewertung mittels einem Therapeutischen Arzneimittel Monitoring basierend auf individuell errechneten Cut-off-Werten gegenüber der qualitativen Urinanalyse Vorteile bietet.

3.3 Diskussion

Bluthochdruck ist eine Volkskrankheit, die eine stetig steigende Zahl an Neuerkrankungen verzeichnet [20]. Trotz der großen Bandbreite an pharmakologischen Behandlungsmöglichkeiten hat sich die arterielle Hypertonie in einem großen Teil der Bevölkerung manifestiert. Da Bluthochdruck eine Hauptursache für kardiovaskuläre Morbidität und Mortalität ist, sind die Prävention sowie Kontrolle von Bluthochdruck durch Lebensstiländerungen [20] oder eine konsequente medikamentöse Therapie umso wichtiger [21,22]. Allerdings ist eine ungenügende Therapietreue in der Bluthochdrucktherapie keine Seltenheit [23], was zu einem gewissen Teil durch den häufig symptomarmen Verlauf und demzufolge fehlenden Leidensdruck unterstützt wird [24]. Hinzu kommt, dass die Arzneistoffe unerwünschte Arzneimittelwirkungen aufweisen. Aufgrund dessen kann das Erreichen einer ausreichenden Blutdruckkontrolle erschwert sein. Bezüglich einer Therapieverfehlung muss zwischen einer fehlenden Adhärenz oder einer TRH als Ursache differenziert werden, da im letzteren Fall nur noch kostspielige, invasive Maßnahme wie eine renale Sympathikusdenervierung oder eine Barorezeptorstimulation als letzte Therapieoption zur Verfügung stehen. Um eine durch eine mangelnde Adhärenz bedingte Pseudoresistenz auszuschließen, sind Verfahren zur Beurteilung der Therapietreue dringend erforderlich. Allerdings weisen alle aktuell zur Verfügung stehenden Methoden Limitationen auf. Während die indirekten Methoden eine große Zeitspanne überblicken, können sie zu keinem Zeitpunkt eine Einnahme garantieren [16,25]. Dementgegen belegen die direkten Methoden eine Einnahme, ermöglichen aber nur die Aussage über einen begrenzten Zeitraum.

Ein Nachteil von rein qualitativen Nachweismethoden ist, dass der letzte Einnahmezeitpunkt nicht abgeschätzt werden kann. Des Weiteren können Sub-

stanzen, die in der verwendeten Matrix (Urin, Serum) über das Dosierungsintervall hinaus nachweisbar sind, ebenso für eine Fehlinterpretation sorgen, wie Substanzen, die nicht über den gesamten Zeitraum eines Dosierungsintervalls nachgewiesen werden können. Um dieser Problematik entgegen zu wirken und Adhärenz zuverlässig belegen oder negieren zu können, wurde eine Methode entwickelt, welche die Gesamtkonzentration (proteingebundene und freie Fraktion) des Analyten im Blut erfasst. Hierzu wurde ein quantitatives flüssigchromatographisch-tandem-massenspektrometrisches Analyseverfahren entwickelt und vollständig nach aktuellen Kriterien validiert [26]. Im Mittelpunkt der Methodik standen hierbei die in Deutschland hauptsächlich verschriebenen Arzneistoffe unterschiedlicher Substanzklassen (AT1-Antagonisten, β-Blocker, Calciumkanal-Blocker, Diuretika) bezogen auf den jährlich erscheinenden Arzneiverordnungsreport. Die entwickelte Methode wurde dann hinsichtlich der zwei Konzepte der unteren dosisbezogenen Konzentration (DRC-SD) und der unteren Grenze des therapeutischen Referenzbereichs (TRR) bei 38 stationären Patienten, die einer überwachten Medikamenteneinnahme unterlagen, auf ihre Zuverlässigkeit überprüft. Hierbei hat sich gezeigt, dass das DRC-SD-Konzept die Adhärenz in allen Fällen bestätigen konnte, während die untere Grenze des TRR im Falle der Diuretika und β-Blocker bei 24,1 % und bezüglich der Calciumkanal-Blocker und AT1-Antagonisten bei 17,9 % der Verschreibungen fälschlicherweise eine Non-Adhärenz signalisierte. Das lässt vermuten, dass diese Bereiche, sofern in der Literatur vorhanden, nicht als Entscheidungsgrenzen für die Beurteilung der Therapietreue geeignet sind.

In einem zweiten Schritt wurden die Adhärenz-Raten ambulanter, nicht-überwachter Patienten mittels des DRC-SD- sowie des TRR-Konzeptes ermittelt und mit dem Ergebnis des Urinscreenings verglichen (*Abbildung 1*). Die in der Literatur beschriebenen Adhärenz-Raten liegen laut Sabaté zwischen 50-70 % [4]. Das deckt sich mit dem Review von Durand et al., welcher nach Ausschluss aller unzuverlässigen Methoden eine mittlere Non-Adhärenz-Rate von 31,1 % ermittelt hat [27]. Die Therapietreue liegt im Falle der unteren dosisbezogenen Konzentration und der Urinanalytik bei den ambulanten Patienten des Krankenhauses Saint-Luc 11-13 % über diesem Wert. Das kann möglicherweise mithilfe des Hawthorne-Effekts (Verhaltensänderung aufgrund des Wissens Teil einer

Studie zu sein) erklärt werden, bei welchem ein Einfluss auf die Medikamenteneinnahme in Form einer gesteigerten Adhärenz bereits beschrieben wurde [28].

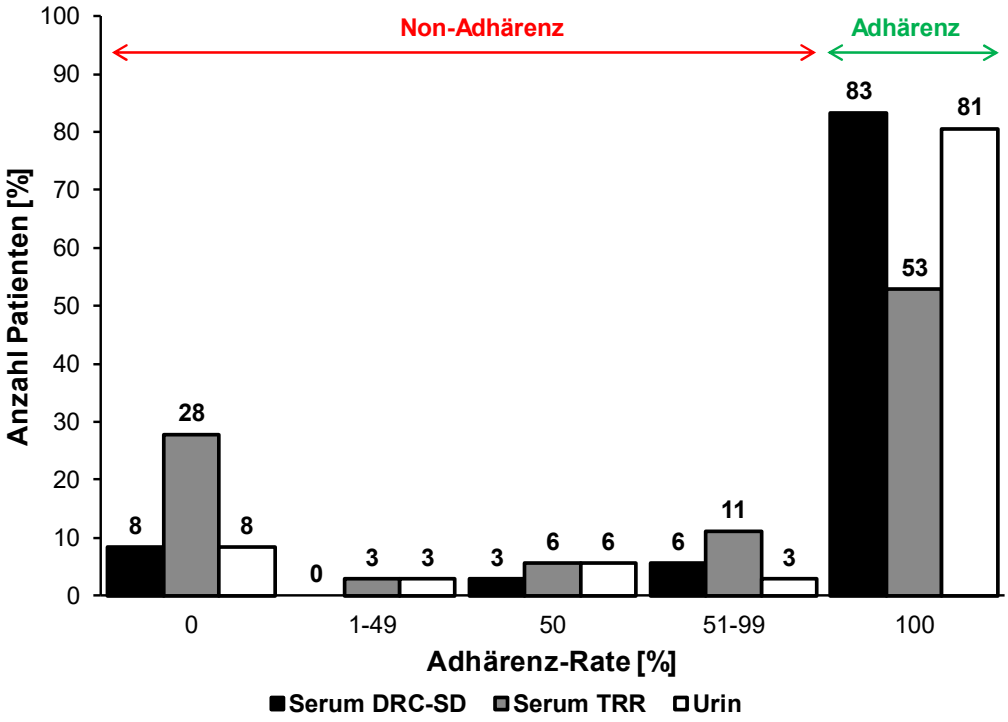

Abbildung 1: Adhärenz-Raten laut Serum- (DRC-SD, TRR) und Urinanalytik der ambulanten Patienten (n=36) des Klinikums Saint-Luc, welche keiner überwachten Medikamenteneinnahme unterlagen

Des Weiteren konnte gezeigt werden, dass die Adhärenz-Raten nicht nur zwischen den Substanzklassen, sondern auch in Abhängigkeit von der verwendeten Methode variieren. Hierbei fällt auf, dass die Adhärenz-Raten der Diuretika und β-Blocker mittels DRC-SD-Konzept als geringer eingeschätzt werden, während diese bei den Calciumkanal-Blockern (+ 5 %) und AT1-Antagonisten (+ 20 %) höher liegen als nach Urinanalytik anzunehmen (*Abbildung 2*). Die bei den Diuretika und β-Blockern geringere und in diesem Patientenkollektiv auch geringste Therapietreue (je 80 %) wurde in der Literatur bereits mehrfach beschrieben [9,29]. Demnach sind das die beiden Substanzklassen mit den niedrigsten Adhärenz-Raten. Das wiederum kann auf die bei den beiden Klassen beschriebenen unerwünschte Arzneimittelwirkungen oder auf das Wissen um diese möglichen Wirkungen (z.B. Diurese, Müdigkeit, Muskelkrämpfe, Erektionsstörungen, Schwindel) [30,31] zurückgeführt werden.

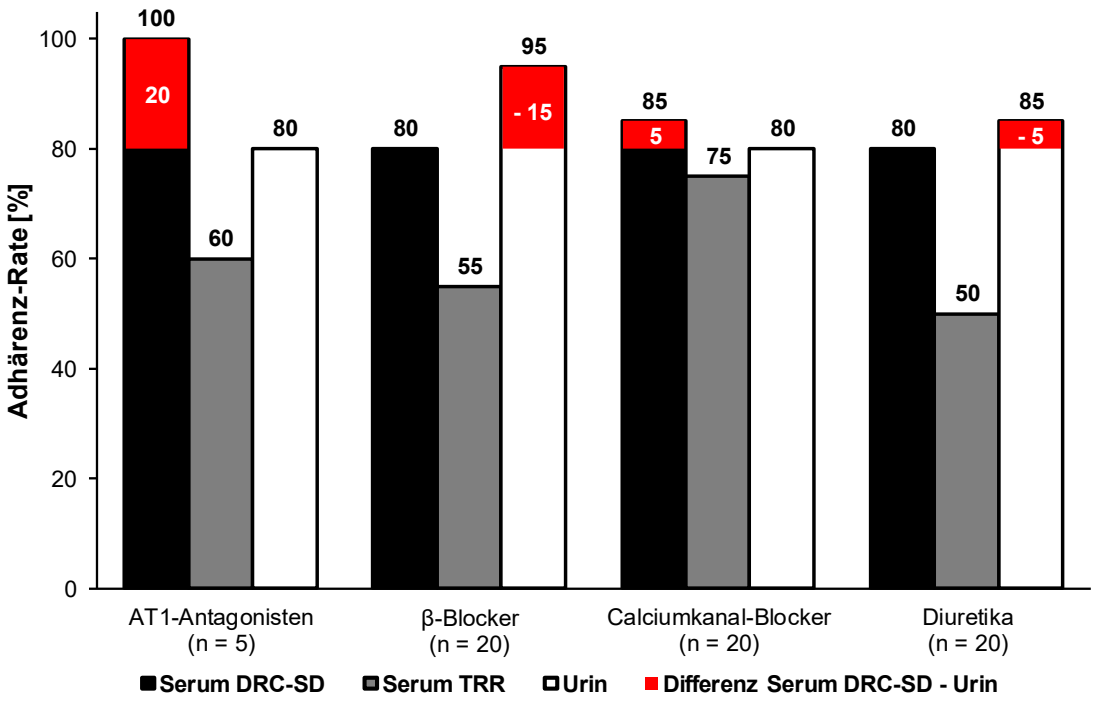

Abbildung 2: Adhärenz-Raten in Abhängigkeit der verwendeten Methodik (untere dosisbezogene Konzentration (Serum DRC-SD), unterer therapeutischer Referenzbereich (Serum TRR) und Urin) aufgeschlüsselt nach den verschiedenen Substanzklassen bezogen auf die ambulanten Patienten (n=36) des Klinikums Saint-Luc, die keiner überwachten Medikamenten-einnahme unterlagen. Der prozentuale Unterschied in den Adhärenz-Raten hinsichtlich der Serum DRC-SD- und der Urin-Methode ist als Differenz in Rot dargestellt.

Bei den Ergebnissen hat sich herauskristallisiert, dass Unterschiede bei der Bewertung der Therapietreue vor allem die β-Blocker Atenolol, Bisoprolol sowie Nebivolol, das Diuretikum HCT, den Calciumkanal-Blocker Lercanidipin und den AT1-Antagonisten Candesartan betrafen. Es hat sich gezeigt, dass in Abhängigkeit der pharmakokinetischen Eigenschaften einer Substanz eine rein qualitative Analyse bei diesen Arzneistoffen zu einer Falschbeurteilung führen kann. Bei einer Einnahme von Atenolol, Bisoprolol und HCT wurden Patienten durch das Urinscreening als adhärent gewertet, während die Methode basierend auf der unteren dosisbezogenen Konzentration eine Non-Adhärenz signalisierte. Gründe für diese offensichtlich falsche Klassifizierung gemäß Urinuntersuchung sind möglicherweise eine hohe Bioverfügbarkeit, eine lange Halbwertszeit und eine überwiegend renale Ausscheidung der Muttersubstanz. In der Folge tendieren

diese Substanzen dazu länger als ein Dosierungsintervall in der biologischen Probe nachweisbar zu sein. Im Gegensatz dazu wurden Patienten, welche die Arzneistoffe Lercanidipin und Candesartan erhielten und im Serum wirksame Konzentrationen aufwiesen, mittels Urinanalytik fälschlicherweise als non-adhärent eingestuft. Diese Arzneistoffe weisen eine niedrige Bioverfügbarkeit auf, werden stark metabolisiert und folglich kaum unverändert renal ausgeschieden.

Die Urinanalyse weist somit für die genannten Arzneistoffe eine Einschränkung auf. Die auf Serumkonzentrationen basierende neu entwickelte Methode könnte in diesen Fällen ergänzend eingesetzt werden. Des Weiteren sollte insbesondere bei der Diagnosestellung einer therapieresistenten Hypertonie (TRH) vor dem Einleiten invasiver Maßnahmen von der Methode mit der unteren dosisbezogenen Konzentration (DRC-SD) Gebrauch gemacht werden.

Die durchgeführten Untersuchungen haben einerseits darauf abgezielt eine quantitative Methode zu entwickeln und eine Bewertungsgrundlage zu schaffen, die dazu dient, eine Adhärenz-Beurteilung bei Bluthochdruckpatienten vorzunehmen. Andererseits sollte gezeigt werden, inwiefern aktuell angewandte rein qualitative Nachweisverfahren zu einer Falschbeurteilung führen können. In den Studien konnte gezeigt werden, dass die Methode, welche auf einem TDM basiert und als Cut-off individuell berechnete dosisbezogenen Konzentration nutzt um die Adhärenz eines Patienten zu beurteilen, eine vielversprechende Methodik darstellt. Die besondere Stärke dieser Methode liegt darin, dass bei Substanzen mit geringer Bioverfügbarkeit, die stark metabolisiert oder kaum renal ausgeschieden werden, genauso wie bei Arzneistoffen, die über einen langen Zeitraum in der untersuchten Probe nachweisbar sind, die Therapietreue der Patienten differenzierter bewertet werden kann.

Allerdings weist auch diese Methodik ihre Grenzen auf. Quantitative Untersuchungen sind im Vergleich zum seit langem etablierten Urinscreening deutlich aufwändiger und es sind bisher nur einige wenige Arzneistoffe validiert. Zudem hängen die gemessenen Konzentrationen der Analyte von unterschiedlichen Faktoren ab. So spielt die genetische Enzymausstattung, aber auch eine TRH, welche durch Malabsorption oder individuelle Unterschiede (z.B. Leber- oder Niereninsuffizienz) in der Ausscheidung bedingt sein kann, eine Rolle. Das kann

zu untypisch niedrigen oder hohen Konzentrationen führen, was dann eine Falschbeurteilung bezüglich der Adhärenz zur Folge haben kann. Andererseits kann bei einer gesicherten Adhärenz diese Wirkstoffspiegelbestimmung auch einen Hinweis auf die genannten Ursachen liefern. Eine grundsätzliche Limitation aller bioanalytischen direkten Verfahren ist, dass immer nur ein sehr eingeschränkter Zeitraum überblickt werden kann [32]. Um das zu kompensieren, könnte die entwickelte TDM-Methodik mit einer indirekten Methode kombiniert werden. Hierfür würde entweder die Methode bei der die Einnahme mittels eines im Medikament enthaltenen Sensors bestätigt [33] wird oder die Überwachung mittels MEMS in Frage kommen.

4 Publikationsübersicht

Die vorliegende publikationsbasierte Dissertation basiert auf den folgenden Originalarbeiten, welche der Auflistung angehängt sind:

Ritscher S, Hoyer M, Wunder C, Obermüller N, Toennes SW: Evaluation of the dose-related concentration approach in therapeutic drug monitoring of diuretics and β-blockers – drug classes with low adherence in antihypertensive therapy. *Scientific Reports:* 9 (1), 15652 (2019) doi:10.1038/s41598-019-52164-y

Impact factor (2018) 4,011

Ritscher S, Georges C, Wunder C, Wallemacq P, Persu A, Toennes SW: Assessment of adherence to diuretics and β-blockers by serum drug monitoring in comparison to urine analysis. *Blood Pressure:* 29 (5), 291-298 (2020) doi:10.1080/08037051. 2020.1763775

Impact factor (2018) 2,292

5 Publikationen

Evaluation of the dose-related concentration approach in therapeutic drug monitoring of diuretics and β-blockers – drug classes with low adherence in antihypertensive therapy

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Evaluation of the dose-related concentration approach in therapeutic drug monitoring of diuretics and β-blockers – drug classes with low adherence in antihypertensive therapy

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Detection of antihypertensive drugs in biological samples is an important tool to assess the adherence of hypertensive patients. Urine and serum/plasma screenings based on qualitative results may lead to misinterpretations regarding drugs with a prolonged detectability. The aim of the present study was to develop a method that can be used for therapeutic drug monitoring (TDM) of antihypertensive drugs with focus on adherence assessment. Therefore, a method for quantification of four diuretics and four β-blockers using high-performance liquid chromatography-mass spectrometric analysis (LC-MS/MS) of combined acidic and basic serum extracts was developed and validated. The method was applied to 40 serum samples from 20 patients in a supervised medication setting (trough and peak serum samples). Literature data on therapeutic concentration ranges, as well as dose-related drug concentrations (calculated from data of pharmacokinetic studies) were used to evaluate adherence assessment criteria. Concentrations were measured for bisoprolol (n = 9 patients), metoprolol (n = 7), nebivolol (n = 1), canrenone (n = 2, metabolite of spironolactone), hydrochlorothiazide (n = 10) and torasemide (n = 8). The measured concentrations were within the therapeutic reference ranges, except for 24% of the samples (mainly β-blockers). In contrast, all measured concentrations were above the lower dose-related concentration (DRC), which appears superior in evaluating adherence. In conclusion, the quantitative analysis of antihypertensive drugs in serum samples and its evaluation on the basis of the individually calculated lower DRC is a promising tool to differentially assess adherence. This method could possibly detect a lack of adherence or other causes of insufficient therapy more reliably than qualitative methods.

Arterial hypertension is a major risk factor for cardiovascular disease worldwide and remains the leading cause of death in the western world[1]. In Europe approximately 4 million people die due to cardiovascular disorders every year[2].

For therapy, drugs of five different classes are typically used (ACE-inhibitors, AT1 antagonists, β-blockers, calcium-channel blockers and diuretics). Non-adherence to a medication plan leads to poor blood pressure control in antihypertensive therapy[3]. This results in frequent visits to the doctor and increased hospital stays[4]. This in return causes greater health care cost[3]. Adherence rates appear to differ between the drug classes. In a meta-analysis by Kronish *et al.* of studies based on medication refill data, poorest adherence was found for diuretics (51%) and β-blockers (28.4%)[5]. This is supported by Gupta *et al.* revealing that the number of prescribed

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drugs alongside with the classes of antihypertensives, especially diuretics, are the main risk factors contributing to non-adherence[6].

There are different methods of adherence assessment (i.e. self-reporting, prescription records, pill count, electronic monitoring systems and toxicological analyses)[7], whereas urine and serum/plasma screenings are the only direct and specific methods[8,9]. However, the qualitative nature may lead to misclassification of adherence in case of excretion much longer than the dosing interval. A diagnostic advancement was achieved by implementing a quantitative method for 21 antihypertensive drugs in serum that was recently published by Gundersen *et al.*[10]. They set up a therapeutic drug monitoring (TDM) system and used pharmacokinetic data to establish calibration ranges to classify measured serum concentrations with respect to adherence.

For the present study a quantitative assay of the main diuretics and β-blockers according to German prescription data was developed and applied to serum of hypertensive patients with confirmed adherence. The concentrations were evaluated with respect to the diagnosis of non-adherence according to two concepts: published reference ranges and the calculated lower dose-related concentration as established for psychiatric TDM[11].

Materials and Methods

Chemicals and reference standards. Reference substances furosemide, torasemide, atenolol, bisoprolol and metoprolol were obtained from Sigma-Aldrich GmbH (Steinheim, Germany). Hydrochlorothiazide (HCT), canrenone and nebivolol, as well as the deuterated internal standards (IS) ketamine-d_4, haloperidol-d_4, diazepam-d_5, quetiapine-d_8, oxazepam-d_5 and methadone-d_9 were purchased from LGC Standards GmbH (Wesel, Germany). HCT-d_2 was obtained from Toronto Research Chemicals (North York, Canada).

Acetonitrile was obtained from Karl Roth GmbH (Karlsruhe, Germany) and ethyl acetate from AppliChem (Darmstadt, Germany). Further chemicals and solvents used were supplied by Sigma-Aldrich GmbH (Steinheim, Germany). All reagents and solvents were either of analytical or LC grade.

Serum samples. Patients (15 males, 5 females) aged 32 to 81 (median 57) years treated with a constant dosing regimen of antihypertensive drugs at the nephrological ward at the University Hospital Frankfurt/Main (Germany) participated in this study. Two blood samples were collected in serum tubes in the morning. The first one shortly before (trough level) and the second approximately two hours after monitored oral administration of the medication (peak level). After centrifugation (2,000 × g for 10 min) the separated serum was stored at −20 C until analysis. For data evaluation information on hospital admission, medication regimen (dose, dosing interval, date of last dose adjustment, co-medication), times of drug intake and of blood sampling were collected. Hospital admission was documented to ensure that steady-state concentrations were reached by the time of blood sampling. The patients' drug ingestion was monitored by the nurses. The study protocol was approved by the competent ethics committee of the Goethe University Frankfurt (reference no. 19/18) and the study was in accordance with the 1964 Helsinki declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Sample preparation. An aliquot of 200 µl serum was transferred to a 2 ml polypropylene reaction tube and 1 ml ethyl acetate, 50 µl internal standard working solution (0.25 ng/µl ketamine-d_4 and methadone-d_9; 0.5 ng/µl haloperidol-d_4, diazepam-d_5, protriptyline-d_3, quetiapine-d_8 and oxazepam-d_5 and 0.05 ng/µl HCT-d_2), 10 µl of acetonitrile (or mixed standard solution or quality control mix, see below) and 50 µl formic acid (10%) were added. After mixing for 2 min and centrifugation at 13,000 × g for 10 min the organic phase was transferred to a silanized glass tube. Another 1 ml ethyl acetate and 50 µl of aqueous ammonia (25%) were added to the aqueous phase followed by mixing for 2 min, centrifugation and transferring to the glass tube. The combined extracts were evaporated at 25 °C with nitrogen using TurboVap LV (Biotage, Uppsala, Sweden). The dry residue was reconstituted with 100 µl of 0.1% formic acid/acetonitrile (80:20, v/v) and transferred to 300 µl glass vials of which 5 µl were analysed.

Calibration standards and quality controls. Human drug-free serum for preparation of quality controls (QC) and calibration standards was provided by healthy volunteers. Stock solutions of furosemide and canrenone were prepared in acetonitrile, whereas torasemide, atenolol, bisoprolol, metoprolol, HCT and nebivolol were dissolved in methanol at concentrations of 1 mg/ml (drug) and kept refrigerated at −20 °C. These were used for preparation of mixed standard solutions and quality controls in acetonitrile. The calibration range was 0.1–20 ng/ml for nebivolol, 2.5–100 ng/ml for bisoprolol, 1–200 ng/ml for metoprolol, 5–1000 ng/ml for atenolol, canrenone, furosemide and HCT and 10–2000 ng/ml for torasemide. The corresponding QC levels (low, medium, high) are listed in Table 1.

Liquid chromatography-mass spectrometry (LC-MS/MS). The analysis was performed on an Agilent (Waldbronn, Germany) LC-MS/MS system consisting of a 1290 Infinity Liquid Chromatograph coupled via JetStream Electrospray Interface (ESI) to a G6460A Triple Quadrupole Mass Spectrometer. Extracts were kept at 20 °C on the autosampler and analytes were separated on a Kinetex® 2.6 µm XB-C18 100 Å LC column (30 × 2.1 mm) plus corresponding guard column from Phenomenex (Aschaffenburg, Germany) at 55 °C.

Gradient elution was performed at a flow rate of 0.4 ml/min using 0.01% formic acid containing 5 mM ammonium formate (A) and acetonitrile containing 0.1% formic acid (B). Gradient elution started with 5% B kept for 0.5 min, increased to 40% B during 2.7 min, maintained 0.3 min, enhanced to 50% B within 0.5 min, held for 0.5 min and increased during 1.5 min to 95% B, maintained 2 min and followed by re-equilibration for 2 min, resulting in a total run time of 8 min.

Source parameters were selected as follows: gas temperature 300 °C, gas flow 11 l/min, nebulizer 45 psi, sheath gas temperature 400 °C, sheath gas flow 12 l/min and capillary voltage 3500 V. Detection was performed in the dynamic multiple reaction monitoring mode (dMRM) according to the mass spectrometry parameters listed in

Analyte	LLOQ (LOD) [ng/ml]	quality control [ng/ml]	intra-day precision [%]	inter-day precision [%]	accuracy [%]	recovery [%]	matrix effects ± SD [%]
Atenolol	0.027	62.5	7.1	7.1	4.2	50.0	92.1 ± 3.1
	(0.007)	250.0	3.9	6.8	5.9		
		625.0	5.3	5.9	−4.0	60.6	85.7 ± 2.7
Bisoprolol	0.006	18.75	5.2	7.4	2.9	85.8	91.8 ± 4.7
	(0.003)	62.5	7.9	7.9	−2.7		
		87.5	4.3	8.5	−3.7	93.6	99.9 ± 5.4
Metoprolol	0.011	12.5	7.1	7.1	1.5	58.3	100.4 ± 2.0
	(0.003)	50.0	7.0	7.0	4.7		
		125.0	6.0	6.0	−4.0	80.1	96.5 ± 4.3
Nebivolol	0.045	1.25	7.7	8.1	0.4	54.5	81.8 ± 7.9
	(0.018)	5.0	5.0	6.1	5.0		
		12.5	7.7	7.7	2.1	85.3	89.7 ± 7.1
Canrenone	0.023	62.5	5.4	6.8	2.5	59.2	92.0 ± 4.1
	(0.008)	250.0	4.3	4.6	6.8		
		625.0	5.0	5.0	−0.9	86.0	94.1 ± 6.8
Furosemide	0.093	62.5	4.6	8.7	6.0	60.0	109.3 ± 14.8
	(0.034)	250.0	6.3	7.2	5.9		
		625.0	6.2	8.0	1.0	83.6	94.1 ± 9.8
HCT	1.592[a]	187.5	4.0	4.0	−1.3	91.3	93.3 ± 9.2
	(0.525)[a]	625.0	3.4	3.4	0.6		
		875.0	2.5	2.7	0.6	92.5	101.2 ± 2.3
Torasemide	0.009	187.5	4.4	8.4	−8.9	66.1	101.7 ± 5.7
	(0.016)	625.0	8.4	8.4	−2.5		
		875.0	5.1	5.8	−3.7	94.5	93.0 ± 6.5

Table 1. Validation data: lower limit of quantification (LLOQ), limit of detection (LOD), intra- and inter-day precision, accuracy, recovery and matrix effects (±SD) measured using the given quality control levels. [a]Determined according to ICH guidelines[33].

Analyte	Retention Time [min]	Precursor Ion [m/z]	Quantifier [m/z] (CE [eV])	Qualifier [m/z] (CE [eV])	Internal standard
Atenolol	1.62	267.2	145.0 (24)	74.1 (20)	Ketamine-d4
HCT	2.04	295.9	268.9 (12)	78.0 (32)	HCT-d_2
Metoprolol	2.79	268.2	74.1 (20)	116.1 (16)	Ketamine-d4
Bisoprolol	3.27	326.2	116.1 (16)	74.1 (24)	Haloperidol-d_4
Torasemide	3.47	349.1	264.0 (12)	183.2 (32)	Methadone-d_9
Furosemide	3.82	329.0	205.0 (16)	78.0 (4)	Oxazepam-d_5
Nebivolol	4.14	406.2	151.0 (32)	103.1 (72)	Quetiapine-d_8
Canrenone	5.44	341.2	107.1 (36)	91.1 (70)	Diazepam-d_5
Internal Standard					
HCT-d_2	2.06	298.0	270.0 (12)		
Ketamine-d_4	2.53	242.1	129.0 (28)		
Quetiapine-d_8	3.67	392.2	258.1 (20)		
Haloperidol-d_4	3.84	380.2	169.1 (20)		
Oxazepam-d_5	4.19	292.1	246.0 (20)		
Methadone-d_9	4.28	319.3	268.1 (8)		
Diazepam-d_5	5.12	290.1	198.1 (32)		

Table 2. Mass spectrometry parameters for the detection of β-blockers and diuretics using LC-MS/MS operated in dynamic MRM mode with two transitions for analytes and one for the corresponding internal standard. Retention times, MRM transitions and collision energies (CE) were as follows.

Table 2. Data acquisition and evaluation was performed using Agilent MassHunter Software (version B.07.00). For identification of analytes a deviation of retention time of less than 0.05 min and a qualifier to quantifier ratio below 20% deviation compared to reference standards were required.

Drug	n	f	τ [h]	CL_t/f [ml/min]	SD [ml/min]	$t_{1/2}$ [h]	Δt [h]	DRC factor [ng/ml/mg]	lower DRC factor [ng/ml/mg]	reference	therapeutic range [ng/ml][17]
Atenolol	30	0.55	24 12	178.3[a]	38.4	6.1	24 12	0.404 0.998	0.356 0.880	[28,34,35]	200–450
Bisoprolol	32	0.88	24 12	337.0[a]	76.2	14.7	24 12	1.111 1.533	0.860 1.186	[36,37]	10–100
Metoprolol tartrate	10	0.55	24 12	1454.6[a]	181.8	4.1	24 12	0.034 0.147	0.030 0.128	[14,38]	20–600[18]
Metoprolol succinate[b]	24	0.45	24 12	2857.2[a]	478.0	3.0	24 12	0.005 0.045	0.004 0.037	[39,40]	
Nebivolol	69	0.12 EM[d]	24 12	7166.7[a]	1611.1	10.3	24 12	0.039 0.063	0.030 0.049	[26,41,42]	<20
		0.96 PM[d]	24 12	307.3[a]	n/a	33.0	24 12	1.738 1.987	n/a n/a		
Canrenone[c]	25	0.25	24 12	1208.0[a]	520.0	14.9	24 12	0.312 0.429	0.178 0.244	[43,44]	100–250
Furosemide	11	0.47	24 12	589.5[a]	150.0	1.9	24 12	0.002 0.066	0.001 0.049	[45]	2000–5000
HCT	58	0.65	24 12	569.4[a]	172.5	10.6	24 12	0.501 0.802	0.349 0.559	[14,26]	40–2000
Torasemide	37	0.79	24 12	43.0	9.8	3.7	24 12	0.819 4.287	0.632 3.310	[46–48]	n/a

Table 3. The data from pharmacokinetic studies refer to healthy volunteers (n = total number of volunteers) with data on bioavailability (f), dosing interval (τ), apparent total clearance (CL_t/f) and its standard deviation (SD), average elimination half-life ($t_{½}$), the mean dose related concentration (DRC) factor with its lower limit for two time intervals between last dose and blood sampling (Δt). The last column cites the therapeutic reference range as retrieved from Schulz *et al.*[17]. [a]Clearance is calculated by dividing the dose by the AUC. [b]Sustained-release formulation. [c]Administered as spironolactone. [d]Genetic polymorphism: data for extensive metabolizers (EM) was used in the present study which differ markedly from those for poor metabolizers (PM).

Method validation. The method was validated according to current guidelines[12]. Statistical evaluation was performed with Valistat 2.0 Software (Arvecon GmbH, Walldorf, Germany).

In order to find appropriate internal standards for the analytes, 41 deuterated medical and illicit drugs were tested. Thus, a broad spectrum of substances with different chemical and chromatographic properties was evaluated. Internal standards were assigned regarding linearity and compensation of matrix effects. Retention time was the decisive factor if deuterated substances yield similar results.

Matrix effects were evaluated by comparing peak areas of spiked extracts with those of standard solutions and recovery by comparing spiked matrix samples to spiked extracts. Both were determined in low and high quality control samples. Each QC sample was measured six times using blank serum samples of different donors.

Selectivity was assessed with human serum samples from eight different drug-free volunteers. Six samples were prepared without (blank samples) and another two by adding internal standard solution (zero samples). To show the absence of interferences serum samples with exogenous substances including typical therapeutic drugs and metabolites, as well as a range of psychoactive substances were analyzed. Sensitivity was assessed by analysing five calibrator concentrations evenly spaced in the range of the expected limit of detection (LOD) and lower limit of quantification (LLOQ) as previously described[13].

Evaluation of linearity was done by six-fold determination in one sequence of seven calibration levels evenly distributed across the calibration range. The calibration was checked for outliers (Grubbs test), homogeneity (Cochran test) and linearity (Mandel test).

For verification of accuracy and precision homogenous pools of low, medium and high quality control samples (relative to calibration range) were prepared by spiking blank matrix and dividing into aliquots. Thereafter two quality controls of each concentration level were measured on eight different days. Results were tested for accuracy (bias ≤15%) and intra- and inter-day precision (relative standard deviation ≤15%).

The analytes are sufficiently stable during long-term storage and during freeze-thaw cycles[10,14–16]. Stability of extracted analytes was tested under autosampler conditions for 72 h. The decrease in concentration of low and high QC samples was checked by repeated injection of an aliquot.

Evaluation of concentrations. The measured concentrations were evaluated by comparison with therapeutic reference ranges as well as with lower limits of calculated dose-related concentrations. Therapeutic reference ranges, indicating therapeutic efficacy and acceptable tolerability, were retrieved from the list of Schulz *et al.*[17] and Repetto *et al.*[18]. If the literature data did not match, the larger reference range was selected for evaluation (Table 3).

The approach to use dose-related concentrations (DRC) consists of comparing measured concentrations with trough serum drug concentrations calculated individually for each patient. To simplify the calculation of expected serum levels, first a factor (DRC factor) was calculated depending on the dosing interval (τ) which is equal to the blood sampling time before the next dose (Δt, 12 or 24 h for both parameters). The necessary pharmacokinetic parameters were retrieved from pharmacokinetic studies on patients without comorbidities, co-medication or

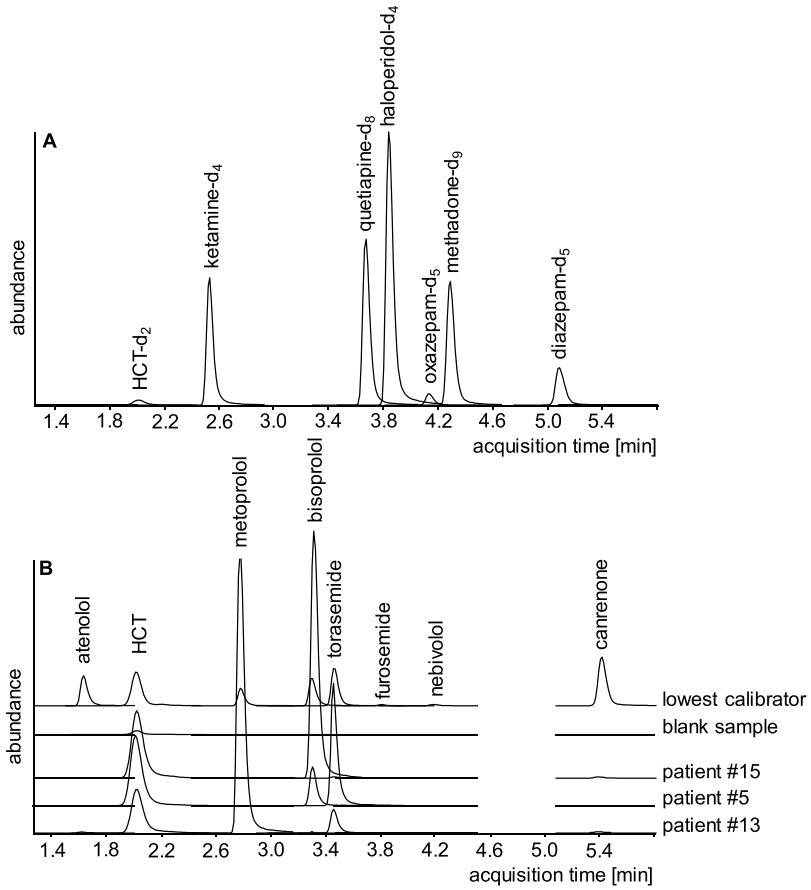

Figure 1. Representative extracted ion chromatograms of internal standards are given in (**A**) and of all analytes in the lowest calibrator, a blank sample, and trough serum samples of patient #15 on HCT (140.5 ng/ml) and bisoprolol (30.2 ng/ml), patient #5 on HCT (139.5 ng/ml), bisoprolol (8.7 ng/ml) and torasemide (439.6 ng/ml) and patient #13 on HCT (108.4 ng/ml), metoprolol (48.5 ng/ml) and torasemide (1752.9 ng/ml) in (**B**), all signals in equal scale.

genetic abnormalities after oral administration (Table 3): bioavailability f, total body clearance CL_t, elimination rate constant k_e.

$$DRC\ factor = \frac{f}{24\ *\ CL_t}\ *\ \frac{\tau\ *\ k_e}{1 - e^{-k_e * \tau}}\ *\ e^{-k_e * \Delta t}\left[\frac{ng}{ml}/mg\right] \quad (1)$$

To cover inter-individual variabilities, the standard deviation (SD) of the apparent total clearance (CL_t/f) as correlate of elimination was incorporated in the calculation (lower DRC factor, based on the concept of Hiemke *et al.*[11]) in a second step.

$$lower\ DRC\ factor = DRC\ factor - \left(\frac{SD}{CL_t/f}\ *\ DRC\ factor\right)\left[\frac{ng}{ml}/mg\right] \quad (2)$$

For each patient the expected trough serum concentration (lower DRC in ng/ml) was calculated by multiplication of the total daily dose in mg with the lower DRC factor. This lower limit of the dose-related concentration was used as a cut-off to evaluate concentrations with regard to adherence assessment.

Results

Method validation. This method based on a two-step liquid liquid-extraction at acidic and basic pH was validated for quantification of four β-blockers and four diuretics. Blank and zero serum samples showed no significant interference in terms of endogenous substances at retention times of analytes or internal standards except that a signal at the retention time of HCT was detected (c.f. chromatogram of a blank sample in Fig. 1) which obviously resulted from non-deuterated HCT present in the HCT-d_2. In this case the LOD and LLOQ were determined based on the standard deviation of the response and the slope according to ICH guidelines (Table 1). Apart from this, neither the spiked nor the analysed samples from patients exhibited signals from other drugs.

Analyte signals were not affected by ion suppression or enhancement. The limit of detection, lower limit of quantification, intra- and inter-day precision, accuracy and recovery as part of the validation procedure are

Patient #	Drug	Daily dose (single) [mg]	lower DRC [ng/ml]	Bisoprolol [ng/ml]	Metoprolol [ng/ml]	Nebivolol [ng/ml]	HCT [ng/ml]	Torasemide [ng/ml]	Canrenone [ng/ml]
1	Metoprolol	50 (25)	1.9		6.6↓/7.3↓				
2	Bisoprolol	2.5	2.2	8.9↓/19.2					
3	Bisoprolol	5 (2.5)	5.9	15.8/16.5					
	HCT	12.5	4.4				100.5/200.2		
4	HCT	12.5	4.4				159.8/264.6		
	Metoprolol	200 (100)	7.4		38.7/42.0				
	Torasemide	20	12.6					50.9/1779.2	
5	Bisoprolol	1.25	1.1	3.3↓/8.7↓					
	HCT	12.5	4.4				44.2/139.5		
	Torasemide	5	3.2					91.3/439.6	
6	Metoprolol	100 (50)	3.7		12.6↓/10.7↓				
7	Metoprolol	47.5	0.2		5.8↓/7.3↓				
8	HCT	25	8.7				286.6/542.5		
9	Bisoprolol	5 (2.5)	5.9	16.4/23.1					
	Torasemide	10	6.3					320.4/1592.4	
10	Spironolactone	25	4.5						47.5↓/100.4
	Torasemide	5	3.2					371.5/1829.2	
11	Bisoprolol	2.5 (1.25)	3.0	15.4/10.9					
	Torasemide	5	3.2					35.1/86.2	
12	Nebivolol	5	0.2			0.4/1.1			
13	HCT	25	8.7				75.2/108.4		
	Metoprolol	190 (95)	7.0		49.4/48.5				
	Torasemide	20	12.6					24.8/1752.9	
14	Metoprolol	200 (100)	7.4		110.8/92.8				
	Torasemide	5	3.2					17.6/1277.9	
15	Bisoprolol	5	4.3	12.9/30.2					
	HCT	12.5	4.4				81.0/140.5		
16	HCT	12.5	4.4				69.5/60.8		
17	Bisoprolol	10 (5)	11.9	21.5/53.8					
18	Spironolactone	25	4.5						25.5↓/44.7↓
	HCT	25	8.7				317.9/606.3		
	Metoprolol	200 (100)	7.4		30.0/24.5				
19	Bisoprolol	10 (5)	11.9	41.1/53.8					
	HCT	12.5	4.4				113.9/167.5		
	Torasemide	20	12.6					39.6/1570.4	
20	Bisoprolol	10	8.6	9.8↓/25.1					
	HCT	25	8.7				15.5↓/96.4		

Table 4. Concentrations of β-blockers and diuretics in serum samples of patients shortly before and about 2 h after observed ingestion (trough/peak). Concentrations below published therapeutic reference ranges are indicated by "↓" (except for torasemide due to missing reference data), no concentrations below the lower DRC (lower DRC factor * daily dose) were observed.

summarized in Table 1. The LLOQs were below expected serum concentrations. The requirements of the Grubbs test (95% significance level), Cochran test (99% significance) and Mandel test (99% significance) were fulfilled and a non-weighted calibration model excluding the origin was used. The calibration curves covered therapeutic ranges and were linear with regression coefficients of at least 0.999. Intra- and inter-day precisions were less than 8.7%, accuracies less than 8.9% (mostly <5.0%) and recoveries higher than 50%. The concentration of the extracted analytes decreased less than 25% during 72 hours of measurement, except of torasemide (48 h), nebivolol (24 h) and canrenone (16 h). Therefore, analysis was always completed within half a day. Representative chromatograms are shown in Fig. 1.

Serum samples. In this study serum of 20 patients on β-blockers and/or diuretics were evaluated. All expected drugs could be quantitated (Table 4) where the trough levels before medication were of special interest. As expected, a marked increase in serum concentrations was observed in the second serum samples representing the time around peak concentrations with a few exceptions (HCT in #16, all metoprolol concentrations).

Reference data on therapeutic plasma levels were used from various sources[17,18] (Table 3) with the exception of torasemide for which no data was available. A high proportion of values were within the expected therapeutic

reference ranges (75.9% of all determined concentrations). None exceeded the higher limit, but canrenone concentrations were mostly (75.0%) below the reported range, as well as 42.9% of metoprolol serum levels. For bisoprolol trough and peak concentrations of patient #5 were both below the therapeutic range (<10 ng/ml[17]) as were the trough samples of patients #2 and #20. In one case a HCT concentration (patient #20, trough) was lower than expected (<40 ng/ml[17]).

In addition to evaluation of concentrations with regard to published reference ranges, the data was also compared with the expected lower limit of the trough serum concentration (lower DRC). This value was individually calculated on the basis of the patient's drug dose and the drug's lower DRC factor (Table 3). The lower DRC includes a diminution by one standard deviation of the apparent total clearance to reflect interindividual variations in excretion. All serum concentrations (trough and peak) of bisoprolol, nebivolol, metoprolol, canrenone, HCT, and torasemide were above these calculated limits.

Discussion

Hypertension is the leading factor for cardiovascular morbidity and mortality[19,20]. Even though there are several pharmacological treatment options, effective high blood pressure management is an ongoing real concern. Since hypertension causes only few symptoms, there is a risk of poor adherence to drugs with unpleasant side effects. Patients not complying with their medication scheme (non-adherence) risk exhibiting a treatment resistant hypertension (TRH). Non-adherence is not easy to diagnose with current methods. Assessment of adherence by direct methods such as toxicological urine or blood analysis is available only occasionally. Based on our detailed previous experience with antihypertensive drug testing in urine[8,21] this methodology is well suited to detect non-adherence, but still has some limitations. One problem is, that a few substances are excreted mainly as metabolites (especially dihydropyridine derivatives[22,23]) and a failure in detection of the drug might lead to misclassification as non-adherent. On the other hand, substances with prolonged excretion may be detectable despite poor adherence (e.g. HCT[24]). As a potential solution it was hypotheticized whether quantitative assays of the drugs in blood would reflect adherence more precisely. In addition, other causes of TRH like malabsorption or individual differences in excretion could be diagnosed more accurately with such an approach.

As a first step a quantitative chromatographic-mass spectrometric target compound analysis procedure was developed and validated. The assay focused on the mainly prescribed diuretics and β-blockers in Germany according to the annual Drug Prescription Report[25]. In a pilot study this method was applied to serum samples obtained from patients in the University Hospital Frankfurt/Main (Germany) with confirmed medication adherence. Results were evaluated with regard to two aspects: (1) does the analytical method yield concentrations that are in accordance with results from published studies and (2) can adherence be confirmed? For this latter aspect the measured concentrations were compared with published reference ranges and with individually calculated cut-off concentrations on the basis of the applied doses.

Concentrations. Of the 20 patients 9 were treated with bisoprolol, which was confirmed in concentrations (trough and peak) of 3.3 to 53.8 ng/ml, 7 with metoprolol (5.8 to 110.8 ng/ml), one with nebivolol (0.36 and 1.08 ng/ml), 10 with HCT (15.5 to 606.3 ng/ml), 8 with torasemide (17.6 to 1829.2 ng/ml) and 2 with canrenone (25.5 to 100.4 ng/ml, active metabolite of administered spironolactone). The measured concentration ranges are in accordance with those found in samples of a routine TDM[10] for bisoprolol (8.14–44.6 ng/ml), metoprolol (3.74–267 ng/ml), canrenone (14.0–91.2 ng/ml) and HCT (7.44–298 ng/ml). However, no data on daily doses or times of blood sampling was provided for a more detailed comparison. The larger concentration ranges in the present data are in agreement with the sampling scheme targeting the minimal (trough) and maximal (peak) concentrations in the patients. The present results also match reported serum concentrations of nebivolol and torasemide[26,27]. Rather high peak and trough concentrations of HCT were found for two patients (#8 and #18, Table 4) which is in agreement with a mean of 673.17 ng/ml that has been reported for elderly hypertensive patients on a daily dose of 25 mg[28]. Therefore, the concentrations measured in the present study are in agreement with published data from patients taking β-blockers and/or diuretics.

Assessment of adherence on the basis of serum concentrations. Adherence assessment is an important part of the diagnosis of treatment resistant hypertension. Several methods have been published to assess patients' adherence[29]. However, so far none of those employed quantitative data in combination with cut-off values.

Adherence rates appear to differ between the classes of antihypertensive medications. Especially low adherence was found for diuretics and β-blockers based on medication refill data[5] which contrasts data from studies using urine or plasma analysis, where these classes were among those with the highest adherence rates[8,30]. In the evaluation of this discrepancy it must be taken into account that toxicological analyses are qualitative in nature and may still be positive even if some time passed since the last drug ingestion. Therefore, it appears necessary to extend toxicological analysis by a quantitative feature and evaluate concentrations in terms of pharmacological activity.

Data on the therapeutic concentration ranges have been reported only for five of the six drugs assayed, torasemide concentrations were therefore excluded from evaluation. None of the concentrations measured exceeded the upper therapeutic limits, but 14 of the total of 58 values (24.1%, 31.0% of the 29 trough values) fell below the lower limit of the concentration range considered therapeutic[17,18]. This affected mainly the β-blockers bisoprolol (especially low doses) and metoprolol, as well as the spironolactone metabolite canrenone (Table 4) and would lead to classification as non-adherent. Since in all cases drug ingestion was monitored this renders the published data as not reliable to differentiate drug ingestion by comparison with the lower limit of the therapeutic reference range. Obviously, reference ranges reflect pharmacologically effective concentrations but cannot be used to evaluate adherence.

For therapeutic drug monitoring (TDM) of antidepressants and neuroleptics this has been improved by Hiemke *et al.*[11]. Expected trough concentrations under steady-state conditions were calculated using a function described by Gex-Fabry *et al.*[31] taking into account dose and dosing interval. In the present study this established concept was applied to evaluate concentrations of antihypertensive drugs. Therefore, lower limits of expected therapeutic concentrations were calculated for different dosing regimens. This based on the concept of Hiemke *et al.*[11] for neuropsychopharmacology where complex dosing regimens were simplified by calculating the total daily dose with a hypothetical dosing interval of 24 h. In the present context this was extended by inclusion of the dosing interval as different doses during a day are rather rare in antihypertensive therapy. This leads to a more appropriate estimation of trough concentrations which are used as cut-offs and are thus more reliable for differentiation of adherence state.

All measured values were above the calculated minimum concentrations expected for the respective dosage schemes. The superiority of this evaluation concept has also been shown for TDM in neuropsychopharmacology[11]. However, limitations of this concept should not be disregarded. For metoprolol, it was striking that serum concentrations of each patient showed hardly any variation from trough to peak. From the patients records it was retrieved that all participants received metoprolol succinate as a sustained-release formulation. From this, a much longer time to maximal concentrations (t_{max}) and smaller peak-trough fluctuations in serum concentrations are expected. Due to the continuous release over 20 hours, a postabsorptive phase, as with the other drugs, does not occur. Since the DRC concept relies on a forecast of the elimination which is different with sustained-release formulations the lower DRC was calculated using pharmacokinetic parameters from appropriate studies (Table 3). Therefore, this concept still allows comparison with measured serum concentrations but an underestimation cannot be excluded. Another limitation that arises in qualitative as well as quantitative methods are substances with low plasma levels and short half-lives. In the present study for instance, doses of furosemide once daily may result in trough concentrations which are very close to the LOD. It is therefore recommended to critically consider the applicability of the quantitative method for adherence assessment in such cases. A peculiarity which remains problematic is that adherence varies over time[32] and the rare ingestion of drugs, especially prior to a doctor's visit (white coat adherence), results in therapeutic serum concentrations which may lead to the false assumption of continuous adherence.

Conclusion

The present proof-of-concept study was performed to evaluate an improved strategy for the assessment of adherence based on quantitative serum drug concentrations. The results with patients on supervised medication adherence show, that established ranges of therapeutic concentrations as far as they are available are not applicable to multi-drug regimens as used for treatment of hypertension. The calculation of lower limits of dose-related concentrations can be used as cut-off values. Concentrations beneath these thresholds may indicate non-adherence or deviations in pharmacokinetics (e.g. malabsorption or rapid drug elimination) which could be used for adaptation of the dosage scheme.

The superiority of evaluating quantitative serum results for assessment of adherence will be investigated in comparison to qualitative results in urine analysis in a study with outpatients without controlled adherence. In addition, the application of this approach to a wider range of antihypertensive drugs is in progress.

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Author contributions

Sabrina Ritscher developed the method, analysed and interpreted the data. Prepared the figures and tables and wrote the main manuscript. Stefan W. Toennes designed the study and contributed to the data analysis and interpretation, the preparation of the figures and tables and the manuscript writing and revision. Milena Hoyer acquired and supervised the patients. Cora Wunder contributed to the method development and validation. Nicholas Obermüller supervised the clinical procedures and revised the manuscript. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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Assessment of adherence to diuretics and β-blockers by serum drug monitoring in comparison to urine analysis

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Assessment of adherence to diuretics and β-blockers by serum drug monitoring in comparison to urine analysis

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Abstract

Purpose: Toxicological screenings for identifying antihypertensive drugs proved to be a useful tool for assessing adherence. However, misinterpretation may occur in case of highly metabolised drugs with low renal excretion, as well as for drugs with a prolonged detectability. The aim of the present study was to compare a recently developed therapeutic drug monitoring (TDM) method based on serum concentrations to an urine drug detection method for assessing adherence in outpatients.

Materials and methods: Corresponding urine and blood samples were obtained at the same time from 26 outpatients without supervised medication. Urine and serum analyses were performed using established high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodologies. Adherence was assumed if drugs were detectable in urine or if serum concentrations were above individually calculated lower dose-related concentrations (DRC) or literature-based therapeutic reference ranges (TRR) used as cut-off, respectively.

Results: The identification of analytes in urine as well as the quantitative serum assay were performed for atenolol (n=6 patients), bisoprolol (n=8), nebivolol (n=6),

canrenone (n=6, metabolite of spironolactone), hydrochlorothiazide (n=12) and furosemide (n=2). On the basis of drug detectability in urine, adherence was assumed in 88 % of prescriptions. In 81 % (DRC) and 50 % (TRR) of the serum analyses the cut-off value was exceeded, which confirms patients' adherence in a lower number. Differences in adherence rates were found in five patients, mainly for β-blockers.

Conclusion: This study suggests that assessment of adherence can be performed more precisely on the basis of serum drug concentrations with individually calculated lower DRC than by using the TRR or qualitative urinalysis.

Key words

LC-MS/MS, antihypertensive therapy, adherence assessment, dose-related concentration, toxicological screenings

1 Introduction

Arterial hypertension is still the leading factor in cardiovascular morbidity and mortality. It is a well treatable disease regarding the wide range of pharmacological treatment options. Non- or poor adherence to therapy makes it difficult to achieve satisfactory blood pressure control, significantly increases the cardiovascular risk [1–3] and may cause treatment resistance [4] to a considerable degree [5]. Non-adherence is estimated in a dimension of 30 to 50 % [5]. Therefore, it is important to exclude non-adherence as a cause of pseudo-resistant hypertension. Drug adherence can either be assessed indirectly (i.e. self-reporting, prescription records, pill count, electronic monitoring systems) or by direct methods (toxicological analyses) [6]. Indirect methods may be affected by manipulations and are therefore limited in their reliability to detect non-adherence [7]. Direct methods are objective but detectability of drugs and conclusions on adherence may be affected by pharmacokinetic aspects.

Reports of adherence rates on the basis of different methodologies show discrepancies. The evaluation of medication refill data by Kronish et al. [8] shows poor adherence for diuretics (51 %) and β-blockers (28.4 %). This is in contrast with data obtained by toxicological screenings where high adherence rates are consistently reported for these classes (β-blockers > 90.4 %, diuretics > 81.9 %) [9,10]. While indirect methods cannot confirm actual drug ingestion, the qualitative nature of

toxicological analyses may lead to overestimation of adherence in case of substances with long excretion times, e.g. of some diuretics and β-blockers. Therefore, setting up a therapeutic drug monitoring (TDM) system using pharmacokinetic data to establish calibration ranges to classify adherence seems to be a diagnostic advancement [11]. Nevertheless, this approach lacks reliable cut-off values to assess adherence. The same applies to a recently published study, in which patients' adherence was assessed based on concentration ratios (CR). These CR cut-offs have not been investigated in patients with monitored drug ingestion and no specific threshold has been defined [12].

Recently, a TDM methodology was further refined by using lower dose-related concentrations (DRC) and therapeutic reference ranges (TRR) as cut-offs to assess patients' adherence [13]. The DRC concept seemed to be reliable in patients with confirmed adherence, whereas using TRR tended to underestimate adherence. The present study was performed to compare this new methodology in a number of outpatients without supervised adherence in comparison to an established qualitative urine drug screening to show relevant methodological limitations.

2 Materials and methods

2.1 Study design

The study was carried out at the hypertension clinic of the Division of Cardiology, Cliniques Universitaires Saint-Luc (UCL, Brussels, Belgium). Patients (11 males, 15 females) aged 36 to 75 (median 61) years, with a median height of 1.71 (range 1.57 to 1.92) m, a median body weight of 79 (range 50 to 111) kg, a median body mass index of 27.7 (range 17.1 to 39.8) kg/m², a median office blood pressure of 138/79 (range 100/50 to 195/133) mmHg and a median eGFR of 66.0 (range 36.0 to 106.5) ml/min/1.73 m² were part of the study. They were treated with in median 3 (range 1 to 7, inter-quartile range 2 to 4) different antihypertensive drugs without supervising their medication intake. From these patients corresponding spot blood and urine samples were obtained at the same visit for toxicological analyses. After centrifugation (3500 x g for 10 min) the serum was separated. All samples were continuously stored at -20°C to guarantee stability of the analytes [13]. For data evaluation information on medication regimen (drug, dose, dosing interval), times of last scheduled drug intake and of blood and urine sampling were provided. The study was approved by the

competent ethics committee and a written informed consent was obtained from the participants on the day of blood and urine sampling.

2.2 Toxicological analyses

The analyses and evaluations were essentially performed as previously described [10,13]. In short, an ethyl acetate extraction (1 ml) of 200 µl aliquots of urine was performed after adding internal standards. The content was mixed for 2 minutes and centrifuged at 13,000 x g for 10 minutes. The organic phase was then transferred to a silanized glass tube and evaporated. For analysis, the dry residue was reconstituted with 100 µl 0.1 % formic acid/acetonitrile (80:20, v/v).

Serum samples were extracted in two steps, where 1 ml acidic ethyl acetate (0.05 % formic acid in ethyl acetate) was first mixed with 200 µl of serum and the supernatant decanted. The aqueous phase was extracted again using 1 ml basic ethyl acetate (1.25 % ammonia in ethyl acetate) and the supernatant was evaporated together with the acidic extract.

The analyses of blood and urine extracts of antihypertensive drugs were done by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) [10,13]. For hydrochlorothiazide (HCT) and furosemide, negative electrospray interface (ESI) mode was used, atenolol, bisoprolol, metoprolol, nebivolol, canrenone and torasemide were analysed in positive ESI mode. The analysis was performed on an Agilent (Waldbronn, Germany) LC-MS/MS system consisting of a 1290 Infinity Liquid Chromatograph coupled via JetStream Electrospray Interface (ESI) to a G6460A Triple Quadrupole Mass Spectrometer. Analytes were separated on a Kinetex® 2.6 µm XB-C18 100 Å LC column (30 x 2.1 mm) equipped with a guard column from Phenomenex (Aschaffenburg, Germany) at 55 °C. Detection was performed in the dynamic multiple reaction monitoring mode (dMRM) with at least one transition for internal standards and two for analytes. Data acquisition and evaluation was performed using Agilent MassHunter Software (version B.07.00). Analytes were considered detected with a deviation of retention time of less than 0.05 min and a qualifier to quantifier ratio below 20 % deviation compared to reference standards.

Urine

For quality control, drug-free urine was used for preparing negative and positive control samples. The latter was spiked with all the reference substances. Analytical detection of the drug or metabolite in urine was considered an indicator of drug adherence.

Serum

For preparation of quality controls, calibration standards and blank sample drug-free serum was used. The calibration ranged from 0.1-20 ng/ml for nebivolol, 2.5-100 ng/ml for bisoprolol, 1-200 ng/ml for metoprolol, 5-1000 ng/ml for atenolol, canrenone, furosemide and HCT and 10-2000 ng/ml for torasemide.

For evaluation of adherence the measured concentrations were compared with the lower limit of published therapeutic reference ranges (TRR) [14,15] and with individually calculated lower limits of the dose-related concentration (DRC), which are considered as promising cut-off for differentiating adherence and non-adherence as recently described by Ritscher et al. [13]. The DRC was individually calculated on the basis of the patient's drug doses and the drug's lower DRC factor (Table 1). The lower DRC is based on the trough serum concentration taking into account the dosing interval (τ) and the time until blood sampling (Δt). Furthermore, it includes a diminution by one standard deviation of the apparent total clearance to reflect interindividual variations in excretion. Concentrations above these cut-offs (TRR, DRC) were considered as indicator of adherence to the respective drug.

3 Results

In this study urine and serum samples of 26 patients on β-blockers and/or diuretics were evaluated. Six patients were treated with atenolol, eight with bisoprolol, six with nebivolol, twelve with HCT, two with furosemide and six with spironolactone (analysed and evaluated as its active metabolite canrenone). In total this were 40 drug determinations (prescriptions, drugs x patients).

3.1 Urine samples

The results of urine analyses (Table 1) suggest adherence to atenolol, bisoprolol and HCT in all patients. In one urine sample nebivolol was not found (16.7 % of nebivolol treated patients), in another one no furosemide was detected (50 %) and in two

samples expected canrenone was absent (33.3 %). Adherence to β-blocker and diuretic medication was assumed in 23 (88.5 %) and non-adherence (one or more prescribed drugs not detected) in three patients (11.5 %).

3.2 Serum samples

Serum concentrations of positive samples ranged from 64.7 to 564.2 (median 153.1) ng/ml for atenolol, 2.5 to 39.8 (median 15.2) ng/ml for bisoprolol, 0.32 to 3.48 (median 0.52) ng/ml for nebivolol, 28.6 to 331.0 (median 75.8) ng/ml for HCT, 43.4 to 221.2 (median 69.6) ng/ml for canrenone (administered as spironolactone) and 22.6 ng/ml for furosemide.

For evaluation [13] one approach is the comparison of serum concentrations with published therapeutic ranges [14,15] Only for nebivolol all values were within the therapeutic reference range (any concentration below 20 ng/ml), but a number of concentrations were below the published ranges (Figure 1). Below were both furosemide values (< 2000 ng/ml), most canrenone concentrations (83.3 %, five patients < 100 ng/ml), a number of atenolol (66.7 %, four patients < 200 ng/ml) and bisoprolol patients (62.5 %, five patients < 10 ng/ml) and some HCT patients (25 %, three patients <40 ng/ml). In only one patient the atenolol concentration exceeded the higher limit (> 500 ng/ml). Using the lower limit of the therapeutic reference range as cut-off for assessing adherence, patients' adherence to the measured drugs would be assumed in only 13 (50 %) of the 26 patients.

The comparison of the quantitative serum data with the lower limits of the dose related concentrations (c.f. Table 1) as indicator of medication adherence suggests that only one patient (50%) adhered to furosemide, four (66.7%) to spironolactone, six (75 %) to bisoprolol, five (83.3%) to atenolol, five (83.3 %) to nebivolol and eleven (91.7%) to HCT. In combination, 21 patients (80.8 %) would be considered as adherent to their β-blocker and diuretic medication.

3.3 Urine vs serum samples

To compare the results of urine and serum samples, the individually calculated lower DRC was used as cut-off criterion which is considered superior to comparison with published therapeutic reference ranges [13–15]. In terms of adherence assessment, the qualitative urine screening result agreed with the quantitative serum assay for

furosemide and canrenone. However, this does not apply to the β-blockers and HCT. Overall one patient (8.3 %) on HCT, one patient (16.7 %) on atenolol, one patient (16.7 %) on nebivolol and two patients (25 %) on bisoprolol were rated as adherent according to the urine screening whereas the quantitative serum results suggested a non-adherence. In contrast, one patient (16.7 %) on nebivolol was classified as non-adherent according to the urinalysis and as adherent regarding the DRC approach (Figure 1).

4 Discussion

As explained in the introduction, hypertension is a well treatable disease but affected by a considerable degree of non-adherence [5]. Indirect methods to assess adherence may be affected by manipulations and are therefore not ultimately reliable [7], but also direct methods may have limitations. One of these are pharmacokinetic aspects which were subject to the present study.

Some substances are metabolised to a large extent and are hardly excreted unchanged via the kidneys (dihydropyridine derivatives [16,17], nebivolol [18]). With toxicological analyses of urine this might lead to misclassification as non-adherent. On the other hand substances with prolonged excretion may still be detectable in urine (e.g. HCT [19]) despite poor adherence, leading to an overestimation of adherence. In order to reduce misinterpretation a quantitative serum assay was evaluated as established for psychiatric TDM [20]. This was shown to be reliable in a number of patients with supervised medication adherence [13] and was now applied to blood samples from outpatients who also provided urine samples allowing the comparison of the performance of both methods. The 26 patients of the present study were treated with in median three different drugs. Since the analyses only looked at β-blockers and diuretics, adherence rates referring to individual patients should not be taken as representative for adherence to all drugs of the medication regimen.

4.1 Concentrations in serum

The concentrations measured above the respective lower DRC (Table 1) are in accordance with those found in the previous evaluation study including only patients with confirmed adherence [13] and with ranges reported in other studies [11,21–23].

4.2 Assessment of adherence on the basis of serum concentrations

The lower DRC was used as cut-off for differentiation of adherence. This value is calculated individually for different dosing regimens based on the concept of Hiemke et al. [13,20]. Overall, eight of 40 values (20 %) were beneath the individual lower limit of the dose-related reference ranges which indicates that the respective drug was not ingested as required (non-adherence) or that the subject exhibited a deviation in typical pharmacokinetics.

As found in the previous study [13] a rather high proportion of the measured concentrations (50 %) failed to comply with published reference data on therapeutic concentrations. Of the 40 determined values 19 (47.5 %) fell below the lower limit of the concentration range considered therapeutic [14,15], because these ranges may not reflect concentrations of low doses as part of complex antihypertension medication regimens [13]. This relates especially to the low doses of the β-blocker bisoprolol as well as the diuretics furosemide and spironolactone (assayed via its active metabolite canrenone) (Table 1). Therefore, the present study suggests that the evaluation of adherence using lower DRCs is the more appropriate approach.

4.3 Assessment of adherence on the basis of qualitative urine analysis results

In the present group of outpatients, adherence rates for the two investigated antihypertensive drug classes according to detection in urine samples were high (95 % for β-blockers and 80 % for diuretics) which is in accordance with results of previous studies using qualitative toxicological analysis to assess adherence [9,10,24]. Overall, in only 3 of 26 patients (11.5 %) non-adherence would have been assumed.

4.4 Discrepancy between urine and serum

Adherence rates differ between classes of antihypertensive medications and between different methods of adherence assessment. Apart from indirect methods qualitative analysis methods may also be imprecise. This is supported by the low adherence as found for diuretics and β-blockers based on medication refill data [8] while data from studies using urine or plasma analysis suggests high adherence rates [9,24]. In the evaluation of this discrepancy it must be taken into account that current toxicological methods are qualitative in nature and may still be positive even if some time passed

since the last drug ingestion due to the high sensitivity of the applied analytical methods. Therefore, the current study was performed to evaluate a more sophisticated and improved method to detect “pseudo-resistant hypertension” caused by non-adherence.

The present data showed that the results of urine and serum analysis did not match in the case of atenolol, bisoprolol, nebivolol and HCT medication. Atenolol may have an extended detectability in urine due to the fact that the drug is predominantly (95 %) eliminated via the kidneys and that the typical elimination half-life of 6-9 h may be increased up to 36 h in renally impaired individuals [21]. This would especially apply to patient #1 (Table 1) with an eGFR of 42.17 ml/min/1.73 m^2 (CKD-EPI) having a moderate reduction of renal function (CKD stage G3b). It is not surprising that atenolol is still detectable in the urine sample even despite occasional non-adherence. Detectability in urine over several dosing intervals can also be expected for bisoprolol and HCT. Approximately 90 % of bisoprolol are bioavailable after oral administration and approximately 50 % of the administered dose is excreted renally unchanged with an elimination half-life of 9 to 12 h [25] and more than 95 % of the absorbed HCT (approx. 70 %) is eliminated unchanged via the kidneys [26,27] with an elimination half-life of approximately 10 h [23]. Therefore, high renal excretion rates may occur despite acute non-adherence and may lead to misinterpretation of adherence (Figure 2). The urine analyses lead to the conclusion that all patients on atenolol, bisoprolol and HCT were adherent to the drugs while the serum analyses in four of 26 cases (15.4 %) revealed concentrations lower than the respective lower DRC. This suggests that one or more dosing intervals have been skipped, indicating at least partial non-adherence. This illustrates, that urine assays are surely capable of reliably detecting non-adherence over several dosing intervals but that serum analyses may be more sensitive in detecting acute omission of ingestion of prescribed drugs.

With qualitative urine assays not only an overestimation of adherence is possible, but also an underestimation in cases of analytes which are highly metabolised and hardly excreted via the kidneys. For instance, nebivolol is highly metabolised and less than 0.5 % of the unchanged drug is eliminated renally [18] and might therefore not be detected in urine assays. In the present urine samples, nebivolol was not detected in one sample (patient #26, Figure 2), which would consequently result in classification of this patient as non-adherent for this drug. In contrast, this patient exhibited a serum concentration above the respective lower DRC for nebivolol (Table 1), suggesting

typical pharmacokinetics and adherence. Though the applied analytical method is highly sensitive and able to detect nebivolol in patients' urine samples [10] (c.f. Figure 2) the results of patient #26 suggest a limitation. An improvement of the urine method could be the inclusion of metabolites [28] but overall, the present approach of analysis in serum is superior in targeting the specific active drugs and may lead to a better assessment of drug adherence. However, the issue remains that adherence is a constantly changing process [29], which cannot be covered by these direct methods. In particular, acute improvements in drug adherence, like the "white coat adherence" phenomenon, cannot be differentiated and may lead to false assumptions.

The present study was performed to evaluate quantitative serum drug concentrations in comparison to a urine assay with respect to adherence assessment. Taken together, these results suggest that drugs with high bioavailability, long half-lives and renal excretion rates as well as highly metabolised substances, which are hardly excreted via the kidneys, may lead to over- or underestimation of adherence via urine analysis. The approach of assessing adherence on the basis of individually calculated lower DRCs as cut-off for serum concentrations might be superior to qualitative urine analysis regarding those drugs. The application of this approach to a wider range of antihypertensive drugs should be investigated in further studies.

5 Disclosure statement

The authors declare that they have no conflict of interest.

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Table 1 Serum concentrations and qualitative results in urine from corresponding spot samples of outpatients regarding β-blockers and diuretics. Prescribed dosages and times (∆t) between blood/urine sampling and last dose (in parentheses) are given. Concentrations below or above published therapeutic reference ranges are indicated by "↓" or "↑", concentrations below the lower DRC (c.f. [13]) are indicated by "*". Positive results of the urine assay are marked by "+", negative results by "-".

Patient #	**Drug**	**Total daily (last) dose [mg]**	**∆t [h]**	**Lower DRC [ng/ml]**	**Atenolol [ng/ml]**	**Bisoprolol [ng/ml]**	**Nebivolol [ng/ml]**	**HCT [ng/ml]**	**Furosemide [ng/ml]**	**Canrenone [ng/ml]**
1	Atenolol	100 (50)	12	88.0	16.7↓* / +					
	Spironolactone	25	24	4.5						0 ↓* / -
	Furosemide	80	24	0.72					0 ↓* / -	
	HCT	12.5	24	4.4				4.3 ↓* / +		
2	Spironolactone	50	24	8.9						0 ↓* / -
	HCT	12.5	24	4.4				38.6↓ / +		
3	HCT	25	24	8.7				230.0 / +		
4	Bisoprolol	10	24	8.6		39.8 / +				
	HCT	25 (12.5)	12	14.0				54.1 / +		
5	Atenolol	50 (25)	6	87.4	564.2↑ / +					
6	Nebivolol	5	6	0.5			0.9 / +			
7	HCT	25	6	28.5				142.8 / +		
8	Atenolol	50	12	44.0	218.4 / +					
9	Bisoprolol	5 (2.5)	4	8.6		24.4 / +				
	Spironolactone	50	24	8.9						60.4↓ / +
	HCT	12.5	4	16.2				59.7 / +		
10	Spironolactone	100	4	45.1						221.2 / +
11	HCT	12.5	2	18.5				75.8 / +		
12	Bisoprolol	2.5	8	4.6		3.3↓* / +				

13	Nebivolol	5	2	0.7			0.5* / +			
14	Spironolactone	25	6	10.3						43.4↓ / +
	HCT	25	6	28.5				99.3 / +		
15	Bisoprolol	10	6	20.1		23.9 / +				
	HCT	50	6	57.0				331.0 / +		
16	Atenolol	25	24	8.9	153.1↓ / +					
17	Nebivolol	5	24	0.2			3.5 / +			
18	Atenolol	50	24	17.8	135.1↓ / +					
	Furosemide	40	24	0.04					22.6↓ / +	
19	Bisoprolol	2.5	24	2.2		6.6↓ / +				
20	Atenolol	50	24	17.8	64.7↓ / +					
21	Bisoprolol	2.5	24	2.2		5.8↓ / +				
22	Bisoprolol	5	24	4.3		1.5↓* / +				
	HCT	12.5	24	4.4				40.1 / +		
23	Nebivolol	5	24	0.2			0.4 / +			
24	Nebivolol	5	24	0.2			0.5 / +			
	HCT	12.5	24	4.4				85.8 / +		
	Spironolactone	25	24	4.5						78.7↓ / +
25	Bisoprolol	2.5	24	2.2		2.5 ↓ / +				
	HCT	25	24	8.7				28.6↓ / +		
26	Nebivolol	5	24	0.2			0.3 / -			

Figure 1 Proportion of patients considered adherent according to the lower dose-related concentration (DRC, serum), the lower limit of therapeutic reference ranges (TRR, serum) and the detectability in the qualitative urinalysis. Data are given for each antihypertensive drug and for the total adherence.

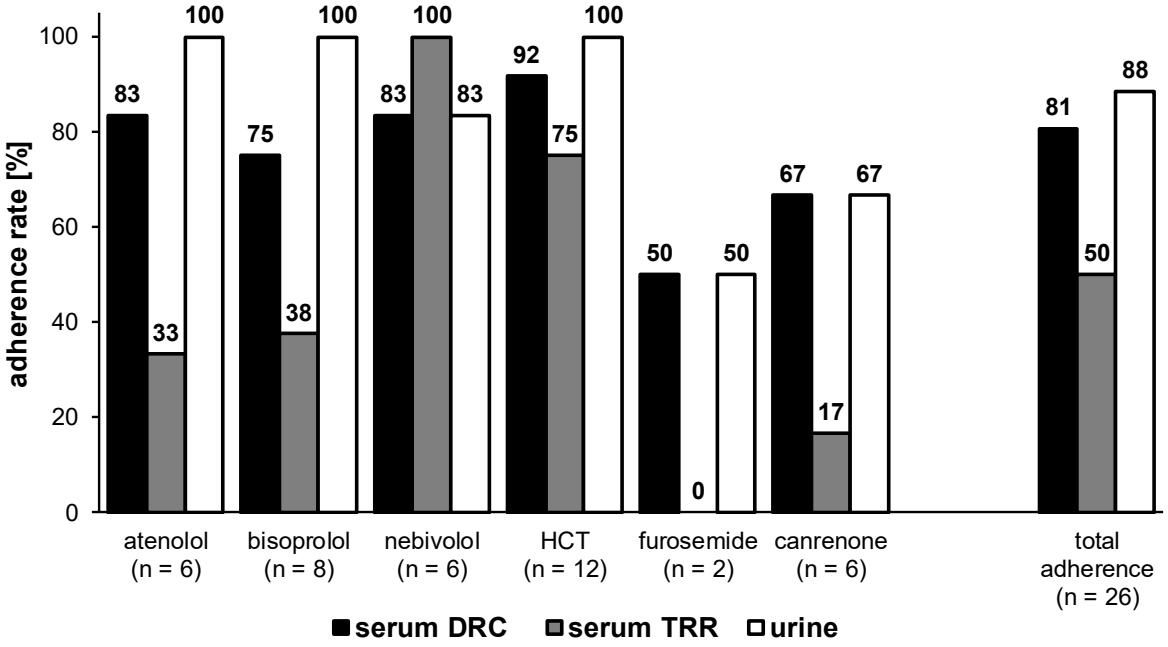
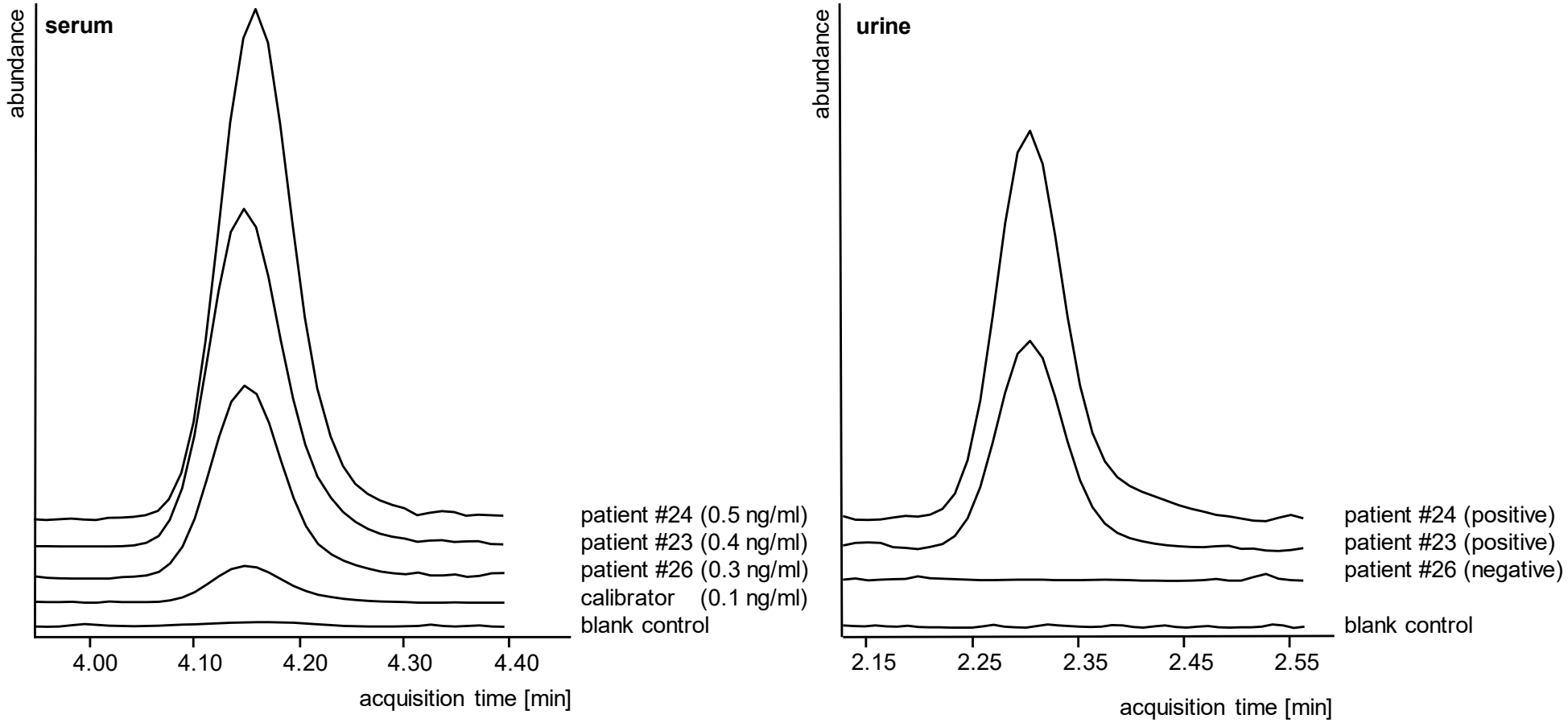


Figure 2 Analytical data (extracted ion chromatograms, equally scaled for direct comparison) of nebivolol in serum and urine. Patient #23, #24 and #26 exhibited similar serum concentrations and were considered adherent according to the DRC approach. In comparison, the urinalysis was considered positive for patients #23 and #24 but negative for patient #26.



6 Darstellung des Eigenanteils

Zur Quantifizierung des Eigenanteils wurde zwischen vollständig (alle Arbeitsschritte im Austausch mit Kollegen), überwiegend (Mehrheit der Arbeitsschritte) und gleichwertig (zu gleichen Teilen) differenziert.

Der Eigenanteil lässt sich wie folgt aufgliedern:

Vollständig:
Literaturrecherche, theoretische Konzeption inklusive Fragestellung, Erstellung des Ethikantrages, Entwicklung und Validierung der LC-MS/MS-Methode, Erstellung der Konzepte, Weiterentwicklung der Urinscreening-Methode, Datenauswertung basierend auf den verschiedenen Konzepten, die Diskussion und Interpretation der Ergebnisse

Überwiegend:
Erstellung der Manuskripte und deren Revision

Gleichwertig:
Studienplanung sowie die Kooperationen mit dem Universitätsklinikum Frankfurt am Main und dem Klinikum Saint-Luc

Die Aufklärungsgespräche, die Blutentnahmen und die Erhebung der Daten, welche die Patienten des Frankfurter Uniklinikums betreffen, erfolgten als Teil der Kooperation durch Frau Dr. Milena Hoyer (geb. Opper). Für den Ethikkommissionsantrag, die Aufklärungsgespräche, die Erhebung der Patientendaten und die Blut- und Urinentnahmen, welche vom Klinikum Saint-Luc bereitgestellt wurden, ist Coralie Georges verantwortlich gewesen.

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Anhang

Im Anhang befindet sich das Patientenblatt, welches für die Datenerfassung bei den stationären Patienten des Universitätsklinikums Frankfurt am Main verwendet wurde. Des Weiteren sind die Validierungsdaten für die verschiedenen Substanzen, die Daten, welche zur Berechnung der unteren dosisbezogenen Arzneimittelkonzentration dienten, sowie auch die therapeutischen Referenzbereiche gelistet.

Patientenblatt: Pilotstudie zur Beurteilung der Adhärenz bei Antihypertensiva-Therapie mittels TDM

Klinik: Universitätsklinikum Frankfurt am Main

Station: A4 (Nephrologie)

Patientendaten

Patientencode: **AHT __ __ __** (fortlaufende dreistellige Nummer)

Name, Vorname:

Geschlecht: ☐ männlich ☐ weiblich

Geburtsdatum:

Größe: cm

Gewicht: kg

Diagnose:_____

Compliance gewährleistet durch:

☐ Überwachung auf Station ☐ Patientengespräch ☐ Angehörige

☐

Medikation

Aktuelle Arzneimittel (FAM/ Wirkstoff, ggf. Retardierung)	**Stärke**	**Dosierung (z.B. 1-0-0-0)**

Probenentnahme

Blutentnahmezeitpunkte bei einmaliger Dosierung: ➢ *Entnahme über den peripheren Venenkatheter* ➢ *mind. 30min. Abstand zur letzten Infusion* ➢ *max. 7,0ml Blut*	
*Tabletteneinnahme **morgens** (Datum + Uhrzeit):*	
Talspiegel (vor der Einnahme)	*ca. 2h nach der Einnahme*

Ansprechpartner
Prof. Dr. S. Tönnes; Dr. C. Wunder

Abbildung 1: Patientenblatt, welches zur Erfassung der Patientendaten, des Medikationsplans, den Zeitpunkt der Tabletteneinnahme und Probenentnahme der stationären Patienten im Universitätsklinikum Frankfurt am Main diente.

Tabelle 1: LC-MS/MS-Parameter für den Nachweis der Antihypertensiva im dynamischen MRM-Modus mit zwei Übergängen für Analyten und einem für den entsprechenden internen Standard. Retentionszeiten, MRM-Übergänge und Kollisionsenergien (CE) waren wie folgt.

Analyt	**Retentionszeit [min]**	**Precursor Ion [m/z]**	**Quantifier [m/z] (CE [eV])**	**Qualifier [m/z] (CE [eV])**	**Interner Standard**
Atenolol	1,62	267,2	145,0 (24)	74,1 (20)	Ketamin-d4
HCT	2,04	295,9	268,9 (12)	78,0 (32)	HCT-d_2
Metoprolol	2,79	268,2	74,1 (20)	116,1 (16)	Ketamin-d4
Bisoprolol	3,27	326,2	116,1 (16)	74,1 (24)	Haloperidol-d_4
Torasemid	3,47	349,1	264,0 (12)	183,2 (32)	Methadon-d_9
Furosemid	3,82	329,0	205,0 (16)	78,0 (4)	Oxazepam-d_5
Amlodipin	4,07	409,2	238,0 (4)	294,1 (4)	Lorazepam-d_4
Nebivolol	4,14	406,2	151,0 (32)	103,1 (72)	Quetiapin-d_8
Candesartan	4,41	441,2	263,1 (4)	192,1 (24)	Lorazepam-d_4
Telmisartan	4,99	515,2	276,1 (48)	497,2 (32)	Diazepam-d_5
Valsartan	5,01	436,2	291,1 (12)	235,1 (12)	Diazepam-d_5
Canrenon	5,44	341,2	107,1 (36)	91,1 (70)	Diazepam-d_5
Lercanidipin	5,57	612,3	100,1 (36)	280,2 (20)	Nordiazepam-d_5
Interner Standard					
HCT-d_2	2,06	298,0	270,0 (12)		
Ketamin-d_4	2,53	242,1	129,0 (28)		
Quetiapin-d_8	3,67	392,2	258,1 (20)		
Haloperidol-d_4	3,84	380,2	169,1 (20)		
Oxazepam-d_5	4,19	292,1	246,0 (20)		
Methadon-d_9	4,28	319,3	268,1 (8)		
Lorazepam-d_4	4,31	325,1	279,1 (20)		
Nordiazepam-d_5	4,62	276,1	140,0 (28)		
Diazepam-d_5	5,12	290,1	198,1 (32)		

Tabelle 2: Validierungsdaten der verschiedenen Analyte zur unteren Bestimmungsgrenze (LLOQ), Nachweisgrenze (LOD), Wiederhol- und Laborpräzision, Richtigkeit, Wiederfindung und zu Matrixeffekten, welche anhand der angegebenen Qualitätskontroll-Proben (QC-Proben) ermittelt wurden.

Analyt	**LLOQ (LOD)** [ng/ml]	**QC-Proben** [ng/ml]	**Wiederholpräzision** [%]	**Laborpräzision** [%]	**Richtigkeit** [%]	**Wiederfindung** [%]	**Matrixeffekt** [%]	**Kalibrationsbereich** [ng/ml]
AT1-Antagonisten								
Candesartan	0,200	12,5	4,5	5,1	0,7	61,3	87,9	1,5-300
	(0,080)	50,0	8,0	8,0	-0,8			
		125,0	2,8	4,8	1,3	86,9	91,7	
Telmisartan	0,004	12,5	4,4	4,4	3,0	55,0	97,0	2-400
	(0,001)	50,0	5,9	5,9	0,7			
		125,0	4,1	4,6	-2,8	82,0	98,2	
Valsartan	0,487	125,0	6,2	7,1	-1,5	59,2	96,9	40-8000
	(0,250)	500,0	6,1	6,1	-2,5			
		1250,0	5,5	5,5	-2,1	88,8	88,2	
β-Blocker								
Atenolol	0,027	62,5	7,1	7,1	4,2	50,0	92,1	5-1000
	(0,007)	250,0	3,9	6,8	5,9			
		625,0	5,3	5,9	-4,0	60,6	85,7	
Bisoprolol	0,006	18,75	5,2	7,4	2,9	85,8	91,8	2,5-100
	(0,003)	62,5	7,9	7,9	-2,7			
		87,5	4,3	8,5	-3,7	93,6	99,9	
Metoprolol	0,011	12,5	7,1	7,1	1,5	58,3	100,4	1-200
	(0,003)	50,0	7,0	7,0	4,7			
		125,0	6,0	6,0	-4,0	80,1	96,5	
Nebivolol	0,045	1,25	7,7	8,1	0,4	54,5	81,8	0,1-20
	(0,018)	5,0	5,0	6,1	5,0			
		12,5	7,7	7,7	2,1	85,3	89,7	

Analyt	LLOQ (LOD) [ng/ml]	QC-Proben [ng/ml]	Wiederholpräzision [%]	Laborpräzision [%]	Richtigkeit [%]	Wiederfindung [%]	Matrixeffekt [%]	Kalibrationsbereich [ng/ml]
Calciumkanal-Blocker								
Amlodipin	0,090	18,75	7,7	7,7	-4,6	77,7	94,3	0,5-100
	(0,034)	62,5	8,1	8,7	0,2			
		87,5	5,2	7,2	0,5	89,0	122,1	
Lercanidipin	0,007	0,125	3,5	4,8	7,0	53,7	98,3	0,05-10
	(0,001)	0,50	6,1	6,6	2,3			
		1,25	4,3	7,9	-1,5	78,4	109,8	
Diuretika								
Canrenon	0,023	62,5	5,4	6,8	2,5	59,2	92,0	5-1000
	(0,008)	250,0	4,3	4,6	6,8			
		625,0	5,0	5,0	-0,9	86,0	94,1	
Furosemid	0,093	62,5	4,6	8,7	6,0	60,0	109,3	5-1000
	(0,034)	250,0	6,3	7,2	5,9			
		625,0	6,2	8,0	1,0	83,6	94,1	
HCT	1,592	187,5	4,0	4,0	-1,3	91,3	93,3	5-1000
	(0,525)	625,0	3,4	3,4	0,6			
		875,0	2,5	2,7	0,6	92,5	101,2	
Torasemid	0,009	187,5	4,4	8,4	-8,9	66,1	101,7	10-2000
	(0,016)	625,0	8,4	8,4	-2,5			
		875,0	5,1	5,8	-3,7	94,5	93,0	

Tabelle 3: Daten aus pharmakokinetischen Studien (n = Gesamtzahl gesunder Probanden) zur Bioverfügbarkeit (f), apparenten Clearance (CL_t/f) mit Standardabwiechung (SD) und Eliminationshalbwertszeit ($t_{½}$) dienen als Grundlage zur Ermittlung des Faktors der mittleren dosisbezogenen Konzentration (DRC) und dessen Untergrenze für zwei verschiedene Zeitintervalle (Δt) zwischen der letzten Dosis und der Blutentnahme; die letzte Spalte gibt mit wenigen Ausnahmen den von Schulz et al. [34] publizierten therapeutischen Referenzbereich an.

Analyt	**n**	**f**	**τ** **[h]**	**CL_t/f** **[ml/min]**	**SD** **[ml/min]**	**$t_{1/2}$** **[h]**	**Δt** **[h]**	**DRC Faktor** **[ng/ml /mg]**	**DRC-SD Faktor** **[ng/ml /mg]**	**Ref.**	**Therapeutischer Referenzbereich [ng/ml]**
AT1-Antagonisten											
Candesartan	30	0,15	24 12	1397,6	325,2	11,6	24 12	0,223 0,340	0,171 0,261	[35]	80-180
Telmisartan	51	0,50	24 12	1868,0	872,4	24,6	24 12	0,260 0,312	0,139 0,167	[36-38]	8,5-1299 [36] [b]
Valsartan	24	0,24	24 12	465,7	156,9	9,5	24 12	0,549 0,933	0,364 0,619	[39,40]	800-6000
β-Blocker											
Atenolol	30	0,55	24 12	178,3 [a]	38,4	6,1	24 12	0,404 0,998	0,356 0,880	[41-43]	200-450
Bisoprolol	32	0,88	24 12	337,0 [a]	76,2	14,7	24 12	1,111 1,533	0,860 1,186	[44,45]	10-100
Metoprolol tartrat	10	0,55	24 12	1454,6 [a]	181,8	4,1	24 12	0,034 0,147	0,030 0,128	[46,47]	20-600 [48]
Metoprolol succinat [c]	24	0,45	24 12	2857,2 [a]	478,0	3,0	24 12	0,005 0,045	0,004 0,037	[49,50]	
Nebivolol	69	0,12 EM [e]	24 12	7166,7 [a]	1611,1	10,3	24 12	0,039 0,063	0,030 0,049	[51-53]	<20
		0,96 PM [e]	24 12	307,3	n/a	33,0	24 12	1,738 1,987	n/a n/a		
Calciumkanal-Blocker											
Amlodipin	36	0,64	24 12	783,0	361,1	37,0	24 12	0,702 0,791	0,378 0,426	[54,55]	3-15
Lercanidipin	32	0,10	24 12	78989,9	21787,5	3,4	24 12	0,002 0,004	0,001 0,004	[56]	1,2-13,6 [57]

Analyt	n	f	τ [h]	CL_t/f [ml/min]	SD [ml/min]	$t_{1/2}$ [h]	Δt [h]	DRC Faktor [ng/ml /mg]	DRC-SD Faktor [ng/ml /mg]	Ref.	Therapeutischer Referenzbereich [ng/ml]
Diuretika											
Canrenon [d]	25	0,25	24 12	1208,0 [a]	520,0	14,9	24 12	0,312 0,429	0,178 0,244	58,59	100-250
Furosemid	11	0,47	24 12	589,5 [a]	150,0	1,9	24 12	0,002 0,066	0,001 0,049	60	2000-5000
HCT	58	0,65	24 12	569,4 [a]	172,5	10,6	24 12	0,501 0,802	0,349 0,559	46,52	40-2000
Torasemid	37	0,79	24 12	43,0	9,8	3,7	24 12	0,819 4,287	0,632 3,310	61-63	n/a

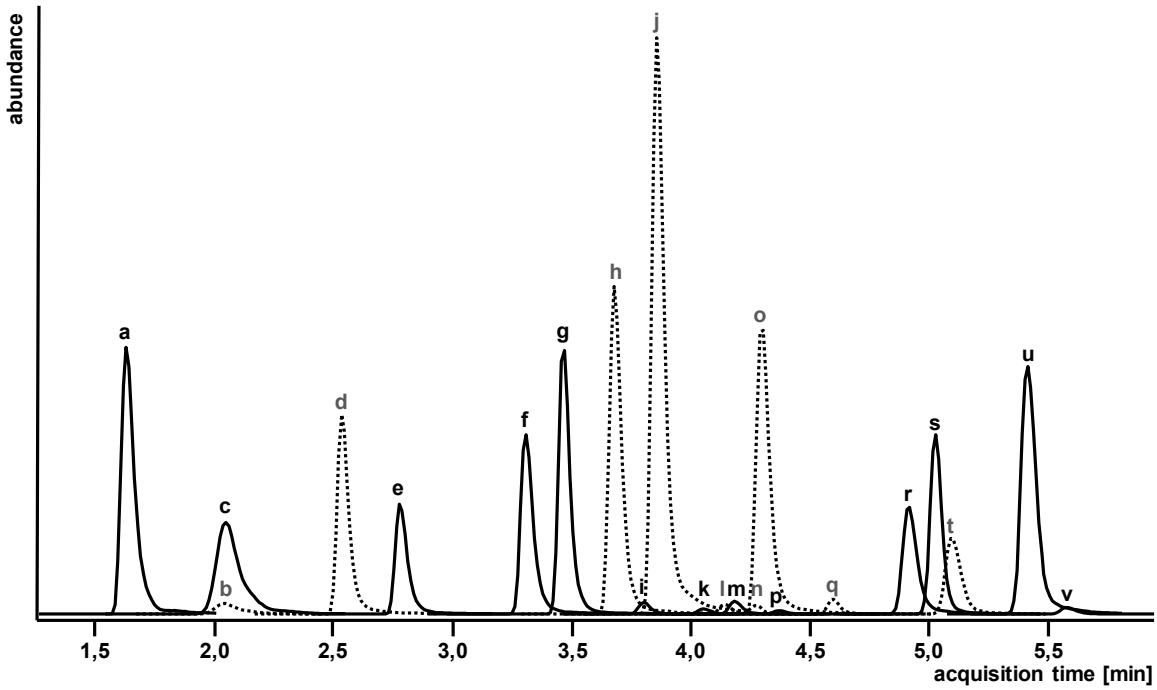
[a] Berechnung der Clearance erfolgte durch Division der Dosis durch die AUC

[b] Bestimmung erfolgte gemäß der AGNP-Konsensus-Leitlinie [64]

[c] Retard-Formulierung

[d] Einnahme erfolgte als Spironolacton

[e] genetischer Polymorphismus: Daten für Schnell-Metabolisierer (EM: extensive metabolizer) wurden zur Auswertung verwendet, welche sich deutlich von denen für Langsam-Metabolisierer (PM: poor metabolizer) unterscheiden.



Atenolol (a), HCT-d_2 (b), HCT (c), Ketamin-d_4 (d), Metoprolol (e), Bisoprolol (f), Torasemid (g), Quetiapin-d_8 (h), Furosemid (i), Haloperidol-d_4 (j), Amlodipin (k), Oxazepam-d_5 (l), Nebivolol (m), Lorazepam-d_4 (n), Methadon-d_9 (o), Candesartan (p), Nordiazepam-d_5 (q), Telmisartan (r), Valsartan (s), Diazepam-d_5 (t), Canrenon (u), Lercanidipin (v)

Abbildung 2: Chromatogramme der extrahierten Ionen von allen quantifizierten Analyten (___) und den verwendeten internen Standards (.....) der TDM-Methodik

Schriftliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die dem Fachbereich Medizin der Johann Wolfgang Goethe-Universität Frankfurt am Main zur Promotionsprüfung eingereichte Dissertation mit dem Titel

Beurteilung der Therapietreue in der antihypertensiven Therapie basierend auf Therapeutischem Drug Monitoring (TDM)

in der Abteilung Forensische Toxikologie des Institutes für Rechtsmedizin unter Betreuung und Anleitung von Prof. Dr. Stefan W. Tönnes ohne sonstige Hilfe selbst durchgeführt und bei der Abfassung der Arbeit keine anderen als die in der Dissertation angeführten Hilfsmittel benutzt habe. Darüber hinaus versichere ich, nicht die Hilfe einer kommerziellen Promotionsvermittlung in Anspruch genommen zu haben.

Ich habe bisher an keiner in- oder ausländischen Universität ein Gesuch um Zulassung zur Promotion eingereicht. Die vorliegende Arbeit wurde bisher nicht als Dissertation eingereicht.

Vorliegende Ergebnisse der Arbeit wurden in folgenden Publikationsorganen veröffentlicht:

Ritscher S, Hoyer M, Wunder C, Obermüller N, Toennes SW: Evaluation of the dose-related concentration approach in therapeutic drug monitoring of diuretics and β-blockers – drug classes with low adherence in antihypertensive therapy. Scientific Reports: 9 (1), 15652 (2019) doi:10.1038/s41598-019-52164-y

Ritscher S, Georges C, Wunder C, Wallemacq P, Persu A, Toennes SW: Assessment of adherence to diuretics and β-blockers by serum drug monitoring in comparison to urine analysis. Blood Pressure: 29 (5), 291-298 (2020) doi:10.1080/08037051.2020. 1763775

_____ _____

(Ort, Datum) (Unterschrift)