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Analytica Chimica Acta 570 (2006) 46–58
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Simultaneous determination of 11 drugs belonging to four different groups in human urine samples by reversed-phase high-performance liquid chromatography method

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Received 5 January 2006; received in revised form 23 March 2006; accepted 1 April 2006
Available online 18 April 2006

Abstract

A new, accurate, sensitive and fast reversed-phase high-performance liquid chromatography (RP-HPLC) as an analytical method for the quantitative determination of 11 drugs in human urine was worked out, optimized and validated. The objects of analysis were imipenem (IMP), paracetamol (PAR), dipyrone (DPR), vancomycin (VCM), amikacin (AMK), fluconazole (FZ), cefazolin (CFZ), prednisolone (PRE), dexamethasone (DEX), furosemide (FUR) and ketoprofen (KET) belonging to four different groups (antibiotics, analgesic, demulcent and diuretic). For HPLC analysis, diode array (DAD) and fluorescence (FL) detectors were used. The separation of analyzed compounds was conducted by means of a LiChroCART® Purospher® C_{18}e (125 mm × 3 mm, particle size 5 μm) analytical column with LiChroCART® LiChrospher® C_{18} (4 mm × 4 mm, particle size 5 μm) pre-column with gradient elution. Analyzed drugs were determined within 20 min. The mobile phase was comprised of various proportions of methanol, acetonitrile and 0.05% trifluoroacetic acid in water. AMK was separated and determined from human urine using *ortho*-phthaldialdehyde–3-mercaptopropionic acid (OPA–3-MPA) as a fluorescent reagent by RP-HPLC-FL. The following retention times for drugs IMP, PAR, DPR, VCM, AMK, FZ, CFZ, PRE, DEX, FUR and KET in human urine were found: 4.01 min, 4.86 min, 6.71 min, 8.14 min, 9.46 min, 10.01 min, 10.90 min, 13.34 min, 14.06 min, 16.03 min and 18.98 min, respectively. Excellent linearity was obtained for compounds in the range of concentration: 0.35–42 μg ml^{-1}, 0.5–45 μg ml^{-1}, 4.5–38 μg ml^{-1}, 0.25–25 μg ml^{-1}, 0.5–35 μg ml^{-1}, 0.25–22 μg ml^{-1}, 0.03–52 μg ml^{-1}, 0.15–25 μg ml^{-1}, 0.25–28 μg ml^{-1}, 0.05–18 μg ml^{-1} and 0.15–35 μg ml^{-1} for IMP, PAR, DPR, VCM, AMK, FZ, CFZ, PRE, DEX, FUR and KET, respectively. The limits of detection (LOD) and limits of quantification (LOQ) for analyzed drugs were calculated in all cases and recovery studies were also performed. Ten human urine samples obtained from patients treated in hospital have been tested. In analyzed samples, one or more drugs from the 11 examined drugs were detected. The concentrations of examined drugs in urine samples ranged between: 1.5–12 μg ml^{-1} of PAR, 5.2–11.5 μg ml^{-1} of DPR, 0.13–9.5 μg ml^{-1} of CFZ and 0.1–8 μg ml^{-1} of FUR. This method can be successfully applied to routine determination of all these drugs in human urine samples.
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Keywords: Drugs; Human urine; Validations; Derivatization; RP-HPLC

1. Introduction

It often happens that patients are treated simultaneously with a few drugs representing different groups. Therefore it is necessary to develop one chromatographic system which could provide simultaneous determination of concurring drugs in the shortest time. In the study, the selection of drugs was made following the frequency of their use in an environment of a postoperative cardiosurgical ward. The combinations of analyzed drugs are in agreement to the ones most often used in the ward and most frequently found together in urine samples. These combinations of drugs can be found in patients urine samples after cardiosurgical interventions whose postoperative course was complicated by infection, sepsis and multi organ dysfunction or failure syndrome. During sample preparation using standards, authors did not examine the combinations of drugs which are not commonly used in clinical practice or which are not recommended to be used simultaneously. No such method

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doi:10.1016/j.aca.2006.04.002

providing simultaneous determination of these drugs in such a short time, in one chromatographic system on biological samples has ever been described before.

Imipenem (IMP) and cefazolin (CFZ) are β-lactam antibiotics, which have potential anti-bacterial activity against a broad spectrum of microorganisms, Gram-positive and Gram-negative bacteria [1–5]. HPLC method has been used for analyses of IMP in biological samples (blood and different rat's tissues) [1,2,6–9] and for pharmacological studies [2].

Broad studies on elaboration procedures determining different antibiotics from cephalosporins and penicillins group in blood were carried out [10]. For particular cephalosporins, different proportional contributions of mobile phase components were adapted [4].

Vancomycin (VCM) as a glycopeptides antibiotic is often used against Gram-positive bacteria, including methicillin-resistant staphylococci [11,12]. Amikacin (AMK) is a semi-synthetic aminoglycoside antibiotic which is derived from kamikacin, and is indicated for intravenous treatment of Gram-negative bacteria [13]. HPLC methods with UV detector [14–17] or with electrochemical detection [18] are often used for indication of VCM in blood or in other tissues. Quantification of VCM and its crystalline degradation product (CDP-1) in human serum by HPLC method with diode array (DAD) detector [19] or Pyr-MS compared with HPLC method have been used [20]. Researched tests comparing HPLC method to fluorescence polarization immunoassay (FPIA) for the analysis of VCM in serum and tissues showed a good correlation between results obtained by HPLC and FPIA [17,21].

Determination of AMK by liquid chromatography (LC) [22,23] mostly requires either pre-column or post-column derivatization by fluorescence (FL) detection. Effective non-derivatization approach to HPLC-fluorescence detection for aminoglycoside antibiotics was based on ligand displacement reaction [24]. Analysis of AMK by LC, with pulsed electrochemical detection on a gold electrode, has also been described [25].

Paracetamol (PAR), dipyrone (DPR) and ketoprofen (KET) are used widely as analgesic and non-steroidal anti-inflammatory drugs [26–28]. Numerous methods have been reported for the analysis of PAR, DPR or KET in pharmaceuticals or in biological fluids. Many HPLC analytical methods exist for the assay of PAR or PAR and its major metabolites in pharmaceutical formulations or biological samples [29–38]. In different methods C_{18} [29,30,32–36,38–41], C_8 [37,42] or C_{18} monolithic [43] columns and DAD detector were used. For the assay of PAR, caffeine, phenobarbital, butalbital, ibuprofen, chlorzoxazone, acetylsalicylic acid and pyridostigmine bromide in the mixtures, the HPLC methods have been reported [40–44].

PAR, caffeine and DPR were separated by HPLC with spectrophotometric detection [44]. Senyuva et al. [45] used a Zorbax SB C_{18} analytical column, mobile phase—methanol/water (80/20, v/v) and DAD detector in order to DPR determination in solid and liquid dosage forms.

Numerous chromatographic methods have been published for quantification of KET in different medias; serum [46], urine [47], plasma [47,48], and also in pharmaceuticals [49,50]. Ameyibor and Stewart [46] used a nonporous octyldecylsilane column with hydroxypropyl β-cyclodextrin as the mobile phase additive in order to KET enantiomers determination in human serum. In other methods, ChiralPak AD [47,48], Spherisorb ODS_2 [50], Kromasil 100 C_{18} [51], Nucleosil C_{18} [52], Shimpack VP-ODS_5 [49], Micra Nonporous ODS [46] analytical columns and DAD detectors were used. In these methods different (isocratics or gradients) flow rates of mobile phase were applied. Simultaneous determination of KET and its degradation products in pharmaceuticals was described by Dvořák et al. [53].

Prednisolone (PRE) and dexamethasone (DEX) are used extensively as anti-inflammatory and immunosuppressive drugs [54,55]. DEX is a synthetic glucocorticoid frequently employed in the diagnosis of adrenal diseases in addition to many inflammatory processes [55]. HPLC methods were used for the detection of PRE and other corticosteroids in urine, serum, plasma or tissues [56–61]. PRE, PRE acetate, cortisol and hydrocortisone in human serum, plasma or urine were separated using a LiChrosorb Si_{60} column or Thermo Hypersil silica analytical column with UV detection [54,56]. For PRE detected in human urine a derivatization by 9-anthroyl cyanide was adapted [62,63]. In other methods, C_{18} columns [57], ion-pair reversed-phase (RP) [64] or monolithic column [65] and DAD detectors were used. A review of the HPLC analysis of steroids, including derivatization methods, has been published by Volin [58] and Nozaki [59]. Mostly before HPLC analysis, analytes are diffused from blood or urine by solid-phase extraction (SPE) [53,57,65,63] or liquid–liquid extractions (LLE) methods [56,64,62]. Liquid chromatography thermospray mass spectrometry (LC-TSP-MS) using isotope dilution [60] and RP-HPLC coupled to tandem mass spectrometry with electrospray ionization (ESI-LC-MS-MS) [61] were also investigated for analysis of PRE, prednisone, DEX and cortisol in human serum.

A sensitive assay was developed for the determination of low levels of DEX in human, swine and rat urine using SPE and HPLC, with UV detection. The separations were made on a C_{18} analytical column [66,67]. Selectivity, sensitivity and precision of HPLC method for simultaneous determination of DEX, hydrocortisone, indomethacin, phenylbutazone and oxyphenbutazone in equine serum appeared appropriate for anti-doping control of racehorses [68]. Separation on C_{18} column [69] or on C_8 column [70] has been developed for the determination of DEX sodium phosphate in nosal- and eye-drops.

Fluconazole (FZ) is an orally active antifungal agent, which is used in the treatment of superficial and systemic candidiasis and in the treatment of cryptococcal infection in patients with the acquired immunodeficency syndrome (AIDS) [71]. Analyses were carried out using reversed-phase methods with application of isocratic mobile phase and the optimal wavelengths of 210 nm [71,72], 260 nm [73,74] and 261 nm [75]. Mostly before HPLC analysis, analytes exhaled from biological fluids by LLE. Most of the previously reported methods published the precision of the calibration data but usually omitted the accuracy for lower concentrations and of the concentration range found in pharmacokinetic and bioavailability studies.

Furosemide (FUR) is a potent diuretic widely used in the treatment of edematous states associated with cardiac, hypertension and chronic renal failure [76,77]. FUR in human urine

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