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Review

A review of high performance liquid chromatographic-mass spectrometric urinary methods for anticancer drug exposure of health care workers

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ABSTRACT

This review describes published high performance liquid chromatography/mass spectrometry (HPLC–MS) methods for the determination of anticancer drugs in human urine as non-invasive tool for monitoring of health care worker exposure to antineoplastic and cytotoxic drugs. HPLC–MS is a sensitive and specific method for analysis of anticancer drugs and their metabolites in biological fluids. In this review, a tabular summary and overview of published HPLC–MS methods are presented, as well as future trends and limitations in this area of research.

1. Introduction

Occupation exposure of healthcare workers to anticancer drugs has been a concern since the early 1980s [1,2]. Workers may be exposed to a drugs throughout their life cycle. These workers include shipping and receiving personnel, pharmacists and pharmacy technicians, nursing personnel, physicians, operating room personnel, environmental services personnel, research laboratory personnel, and workers in veterinary practices where hazardous drugs are used. The number of workers potentially exposed to all hazardous drugs is estimated to be 11 million workers [3].

Recent studies in the U.S. and several other countries show that workplace contamination with antineoplastic drugs is still occurring [4–17]. Contamination of drug preparation and administration areas can lead to exposure of healthcare workers to these drugs as evidenced by contamination of workers' hands and measurement of the drugs in the urine of workers [11,17].

The measurement of anticancer drugs in urine is key in characterizing occupational exposure in health care workers. Anticancer drug levels found in environmental monitoring of workplace surfaces and in the air in drug preparation areas, while reflecting the efficacy of measures to eliminate workplace contamination, these levels cannot be assumed to represent healthcare worker exposure. Since the beginning of formal guidelines and their successful application to reduce exposure of healthcare works to anticancer drugs, the need for sensitive and accurate analytical methods to quantitate exposure are well met by the capability of HPLC–MS methodology. Most anticancer drugs are non-volatile, and thermolabile compounds making gas chromatographic separation and detection unsuitable [18]. Early liquid chromatography detection methods using ultraviolet, fluorescent and electrochemical detection, however sensitive, lacked specificity. Over time, liquid chromatographic separation with mass spectrometric detection has become the preferred method for detection and quantitation of anticancer drugs both in workplace area monitoring and healthcare worker biomonitoring [18].

The current review focuses on HPLC–MS determination of anticancer drugs in the urine of healthcare workers. Earlier analytical methods have been extensively reviewed [19,20] as well as those specifically using LC–MS methodology [18]. The majority of these were developed for pre-clinical and clinical studies. Those for analysis of biological fluids have been developed for blood serum, plasma or urine in clinical animal models or in patients given therapeutic doses of drug. In this review, HPLC–MS methods created for determination of anticancer drugs in urine are summarized in tabular format and highlights of the sample preparation and chromatography techniques used in these methods are briefly described.

2. Tabular summaries of selected methods

Tables 1–4 summarize various HPLC–MS methods reported for the detection and quantification of various antineoplastic drugs in urine of exposed healthcare workers for use in occupational biomonitoring studies. The terminology and abbreviations appearing in these tables indicate sample preparation techniquetographic conditions, and mass spectrometry detectios, chroman modes reported for these methods, and are explained in more detail in the following sections of this review.

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Table 1
LC/MS determination of nitrogen mustard antineoplastic drugs in urine.

Parent drug	Sample preparation	Chromatography	Interface/ Detection	Target analyte	*m/z* of mass transition	Limit of Detection	Reference
cyclophosphamide (CP)	LLE ethylacetate	RP C8/isocratic $CH_3CO_2NH_4$/MeOH 4.6 × 150 mm, 5 µm	ESI/QQQ/MRM$^+$	CP IF	261.2/140.2 261.2/92.0	0.05 µg/L	[21]
cyclophosphamide	LLE ethylacetate	RP C18/gradient CH_3COOH/MeOH 2.1 × 50 mm, 4 µm	ESI/QQQ/MRM$^+$	CP d_6-CP	263.1/142.1 267.1/140.3	0.01 µg/L	[22]
cyclophosphamide	SPE C18 ethylacetate	RP C18/gradient HCO_2NH_4/ACN 3.0 × 150 mm, 3 µm	ESI$^+$/QTrap	CP d_4-CP	261/140 265/140	0.05 µg/L	[23]
cyclophosphamide	SPE C18 ethylacetate/ dichloromethane	RP C18/isocratic CH_3COOH/ACN 3.0 × 100 mm, 2.7 µm	ESI/QQQ/MRM$^+$	CP IF	261/140 261/54	0.07 µg/L	[24]
cyclophosphamide ifosphamide (IF)	salt-assisted LLE ethylacetate sodium borate	RP C8/isocratic HCOOH/ACN 2.0 × 100 mm, 3 µm	ESI/QQQ/MRM$^+$	CP IF PCP	261/154.1 261/140.1 249/164.1	0.1 µg/L 0.1 µg/L	[25]
cyclophosphamide ifosphamide	SPE C18 ethylacetate	RP C8/gradient HCOOH/ACN/MeOH 4.6 × 100 mm, 5 µm	ESI/QQQ/SRM$^+$	CP IF TRP	261.0/140.2 261.0/92.0 323.3/92.0	0.02 µg/L 0.04 µg/L	[26]
cyclophosphamide ifosphamide	SPE C18 MeOH	RP C18/gradient HCOOH/ACN/MeOH 2.1 × 150 mm, 3 µm	ESI$^+$/Ion Trap	CP IF PSL	261/140 261/182 361/343	0.4 µg/L 0.4 µg/L	[27]
cyclophosphamide ifosphamide	LLE dichloromethane	RP C18/gradient HCO_2NH_4/ACN 2.1 × 100 mm, 5 µm	ESI/QQQ/MRM$^+$	CP d_4-CP IF	261/140 264/140 261/92	0.01 µg/L 0.01 µg/L	[38]
cyclophosphamide 4-keto-CP ifosphamide	LLE ethylacetate	RP C18/gradient HCOOH/ACN 3.0 × 250 mm, 3.5 µm	ESI/QQQ/MRM$^+$	CP 4-keto-CP IF d_6-CP	261/140 267/140 275/106 261/154	0.1 µg/L 1.0 µg/L 0.05 µg/L	[28]
cyclophosphamide 4-keto-CP carboxy-CP DCL-CP	LLE MeOH	RP C8/gradient HCOOH/MeOH 3.0 × 100 mm, 5 µm	ESI/QQQ/SRM$^+$	CP 4-keto-CP carboxy-CP DCL-CP d_4-CP	261/140 275/221 293/221 199/171 265/145	5 µg/L 5 µg/L 30 µg/L 1 µg/L	[29]
bendamustine (BM) & phase I metabolites	SPE MeOH	RP C18/gradient HCO_2NH_4/MeOH 2.0 × 150 mm, 4 µm	ESI/QQQ/MRM$^+$	BM BM-IS metabolite 3 metabolite 4	358/228 372/338 374/186 344/354	0.5 µg/L 0.5 µg/L 0.4 µg/L	[30]
bendamustine phase I metabolite	SPE MeOH	Polar RP/gradient HCO_2NH_4/MeOH 2.0 × 150 mm, 4 µm	ESI/QQQ/MRM$^+$	dihydroxy-BM α-DLA	322/304 408/170	1 µg/L	[30]

LC/MS determination of nitrogen mustard antineoplastic drugs in urine.
ACN: acetonitrile, CH_2Cl_2, DCL-CP: *N*-dechloroethyl-cyclophosphamide, α-DLA: α-dansyl-L-arginine, ESI: electrospray ionization, LLE: liquid–liquid extraction, MeOH: methanol, MRM: multiple reaction monitoring, PCP: phencyclidine, PSL: prednisolone, QQQ: triple quadrupole, RP: reversed phase, SPE: solid phase extraction, SRM: single reaction monitoring, TRP: trophosphamide.

2.1. Sample preparation techniques

Successful determination of target analytes by HPLC–MS requires separation of analyte antineoplastic drugs from interfering components found in urine. Proteins, numerous metabolites, salts and other components that make up the urinary sample matrix interfere with the sensitive and specific detection of the target analytes. Salts can suppress the intensity of the analyte signal or similar metabolites may co-elute from the chromatographic column with the target mercapturate. The necessary removal of these interferences make sample preparation as critical to success as any other part of the analysis. A variety of sample preparation techniques have been applied in the methods reviewed. The simplest is protein precipitation by acetonitrile and centrifugation prior to analysis [37]. Most methods use C18 solid phase extraction (SPE) for sample preparation and clean-up. In simple manual SPE techniques, medium in syringes, disks or cartridges are used to extract 1–5 ml of urine. In SPE urine is applied to chromatographic medium, and is pulled through the medium under vacuum pressure. Target analytes are captured in the solid medium, and several volumes of solvent are used to remove sample matrix components. Concentrated and purified analytes then are washed free from the medium using solvent or solvent mixtures. Methods using manual SPE C18 sample preparation for nitrogen mustards (e.g. ifosphamide) used either methanol or ethylacetate as eluants [23,24,26,27,30] while Sottani used methylene chloride/2-propanol mixtures for extraction of anthracyclines (e.g. doxorubicin) from C18 media [34,40].

Sample preparation is often the labor intensive and rate-limiting step in most bioassay methods. SPE media in disk, cartridge and bed forms have been adapted to high-throughput popular 96-multi-well sample plate format when the speed of fully automated analysis is necessary. The convenience of 96- and other multi-well formats is also ideal for rapid development of sample extraction methods [42]. Rule et al., developed a 384-well plate sample extraction and sample handling technique for analysis of methotrexate and 7-hydroxy-