A Sensitive and Selective LC-MS/MS Approach for Simultaneous Determination of 18 Cytotoxic Anticancer Agents in Human Plasma for the Assessment of Occupational Safety

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Purpose

In the last decade, the use of cytotoxic drugs has increased considerably in cancer therapy. Besides curative effects, the cytotoxic agents have potential carcinogenic and mutagenic effects on human. There is an increased concern of the occupational safety and health risks of the nurses in ambulatory oncology settings due to their frequent exposure to a large amount of the cytotoxic anticancer drugs. In order to measure the drug level of 18 frequently used hazardous anticancer agents in the nurse plasma samples and assess the occupational exposure of the ambulatory oncology nurses, we developed a fast, sensitive and reproducible liquid chromatography tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of the 18 anticancer drugs in human plasma

Methods

Materials and sample preparation

Drug standards were purchased from Sigma-Aldrich. Fresh plasma was obtained from healthy volunteer. Formic acid and LCMS grade Acetonitrile were purchased from Fisher Scientific. NSLS00503, used as internal standard (IS), was made from UM research group. Deionized water was used throughout the study.

To precipitate the proteins in human plasma, 300 µL of acetonitrile containing 10 ng/mL IS were added to 100 µL of human plasma sample. The extracts were centrifuged and the supernatant was transferred to the autosampler vials for LC-MS/MS analysis. 5 µL of the supernatant was injected. Blank human plasma (Bioreclamation LLC) was used to prepare spiked calibration standards. The concentrations of the calibration standards were 0.25, 0.5, 1.0, 2.5, 5.0, 10, and 25 ng/mL.

Instruments

LC separation was achieved on a Shimadzu UFLC SIL-20 with a Waters XBridge™ C18 column(3.5µ m, 4.6 X 150mm). Gradient elution was used with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in Acetonitrile). Table 1 showed the detailed gradient for mobile phase B during the entire run time (17 min). The column oven was set at 35 °C.

Table 1. LC Gradient for the Anticancer Drug Separation

Time (min)	0	2.0	3.0	4.0	11.0	11.1	13.5	13.6	17
Solvent B %	0	0	5	20	70	95	95	0	0

MS/MS detection was carried out on a AB Sciex 5500 QTRAP® mass spectrometer equipped with a Turbo V ion source. Multiple reaction monitoring transitions and optimized transitions for each anticancer agent were listed in Table 2.

Results

Separation of Anticancer Drugs

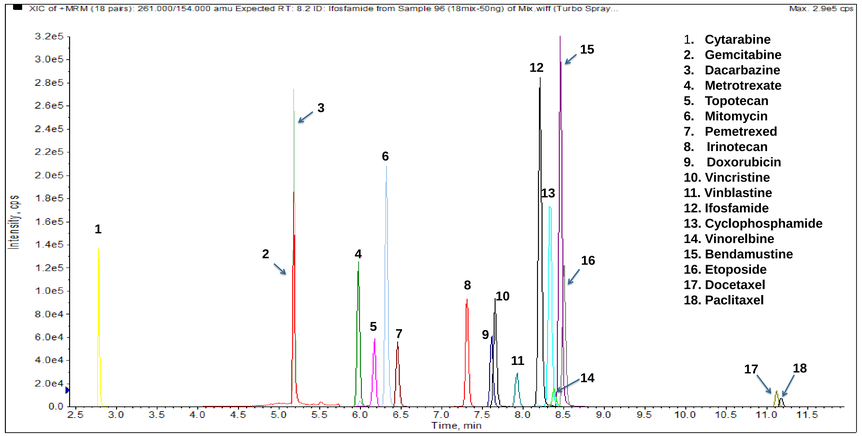

Fig.1. Overlaid MRM chromatograms of 18 selected drugs in a 17 minutes LC run.

Anticancer Drugs Quantitation in Spiked Human Plasma

Table 2. LC-MRM Parameters, Precision , Accuracy, and Recovery of the Anticancer Drugs

Substance	Q1 (amu)	Q3 (amu)	CE (V)	Retention time(min)	% CV (10ng/mL)	% Accuracy	Recovery (%, n=3)	Linear Range (ng/mL)
Cytarabine	244.1	122.1	20	2.78	6.8	93.7	75	0.5 - 25
Gemcitabine	264.1	122.1	23	5.18	11.8	100.4	73	0.5 - 25
Dacarbazine	183.1	123.0	23	5.18	10.4	99.5	122	0.5 - 25
Metrotrexate	455.2	308.0	30	5.98	15	101.0	76	0.5 - 25
Topotecan	422.2	377.0	32	6.17	29	102.4	93	0.5 - 25
Mitomycin	335.2	242.0	19	6.32	12.7	98.7	119	0.5 - 25
Pemetrexed	428.2	281.0	28	6.46	13.3	102.0	32	0.5 - 25
Irinotecan	587.3	502.3	43	7.31	7.0	98.3	124	0.5 - 25
Doxorubicin	413.2	353.2	18	7.62	11.3	96.3	126	0.5 - 25
Vincristine	544.2	397.1	33	7.66	13.2	100.0	120	0.5 - 25
Vinblastine	406.3	271.6	34	7.93	10.7	91.5	111	0.5 - 25
Ifosfamide	261.0	154.0	32	8.21	10.5	100.6	127	0.5 - 25
Cyclophosphamide	261.1	139.9	35	8.34	13.1	99.2	127	0.5 - 25
Vinorelbine	390.0	626.3	23	8.39	12.2	100.7	112	0.5 - 25
Bendamustine	358.0	304.1	40	8.46	28	89.2	121	0.5 - 25
Etoposide	589.1	229.1	23	8.51	13.3	96.3	137	0.5 - 25
Docetaxel	808.3	527.2	16	11.12	13.7	105.7	116	0.5 - 25
Paclitaxel	854.3	569.3	16	11.18	12.3	96.6	115	0.5 - 25

Results and Conclusion

Table 3. Comparison of Extraction Efficiency between SPE and Protein Precipitation

Substance (12.5ng/mL)	SPE	Protein precipitation	Substance (12.5ng/mL)	SPE	Protein precipitation
Cytarabine	No	7.90E+04	Doxorubicin	1.00E+05	8.90E+04
Gemcitabine	No	7.60E+04	Vinblastine	2.40E+04	2.40E+04
Dacarbazine	1.50E+05	1.15E+06	Ifosfamide	1.50E+05	1.30E+05
Metrotrexate	1.80E+05	2.60E+04	Cyclophosphamide	1.30E+05	1.20E+05
Topotecan	4.00E+04	2.80E+04	Vinorelbine	4.30E+04	3.50E+04
Mitomycin	8.80E+04	7.50E+04	Bendamustine	5.50E+04	4.90E+04
Pemetrexed	1.50E+04	1.73E+03	Etoposide	9.00E+04	7.00E+04
Irinotecan	4.80E+04	4.80E+04	Docetaxel	9.00E+03	7.50E+03
Vincristine	5.90E+04	6.50E+04	Paclitaxel	2.20E+04	2.00E+04

Calibration Curves

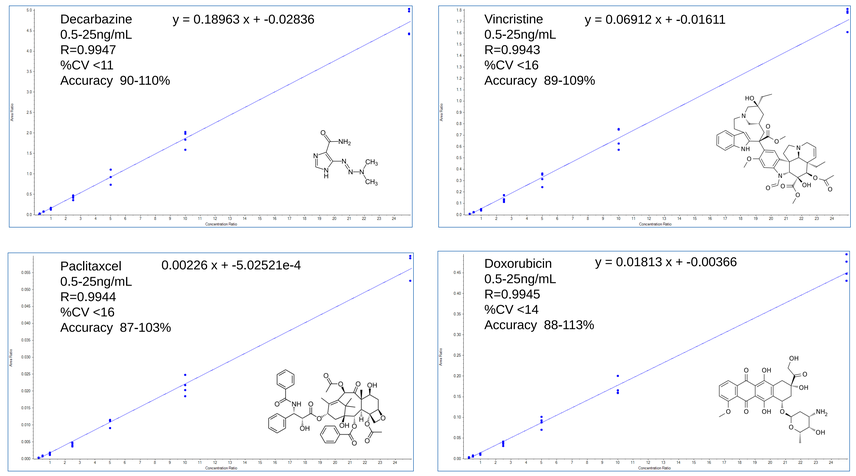

Fig.2. Representative calibration curves for anticancer drug quantitation. Good linearity was obtained for all the analytes in the concentration range of 0.5-25 ng/mL (R>0.99) with high accuracy, precision and reproducibility.

Conclusion: A sensitive LC-MS/MS method was developed and validated for the simultaneous detection and quantification of the 18 commonly handled cytotoxic drugs in human plasma. The method has been applied to monitor the drug level in the plasma from the nurses in the ambulatory oncology settings. The results will be useful to assess the drug exposure and occupational safety of the oncology nurses. The method can also be useful for other applications in biological and environmental monitoring. The research is funded by the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention R01 OH 010582–01.