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LC/MS Application Note

On-line automated protein precipitation preparation followed by LC-MS/MS analysis and LDTD-MS/MS cross validation

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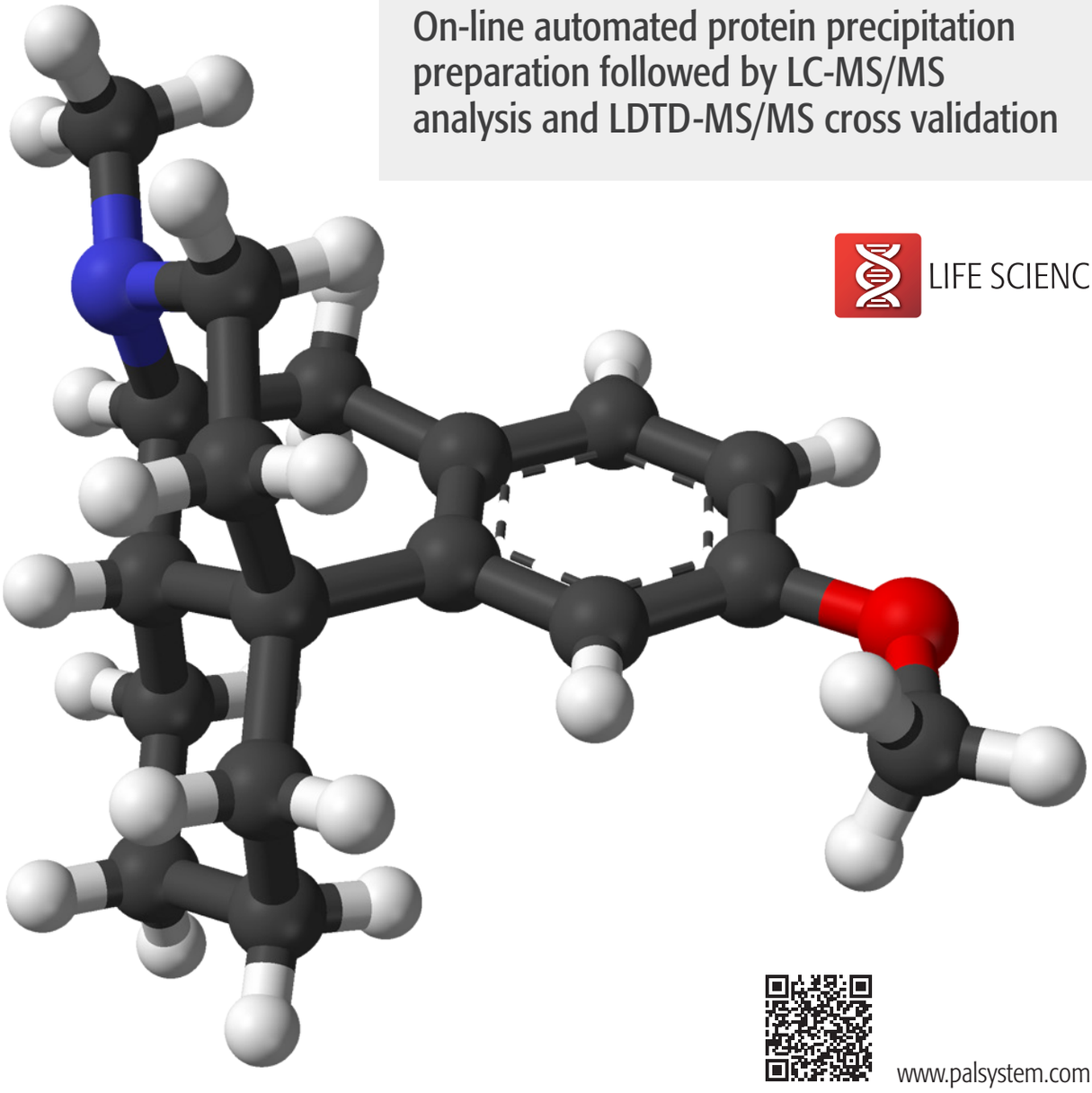

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On-line automated protein precipitation preparation followed by LC-MS/MS analysis and LDTD-MS/MS cross validation

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Introduction

To minimize contamination or inversion during the preanalysis sample preparation, a new on-line sample preparation system is evaluated. The Robotic Tool Change (RTC) is an on-line extraction and injection system. Complex automated sample preparation steps such as dilution, derivatization, mixing, liquid/liquid extraction, and protein precipitation can be performed prior to injection. Using the RTC system, an on-line protein precipitation of plasma samples followed by LC-MS/MS analysis was performed. During the same process, samples were also deposited in LazWell plates for Laser Diode Thermal Desorption analysis (LDTD-MS/MS). The same on-line sample preparation steps were used to do cross validation of LC-MS/MS versus High throughput LDTD-MS/MS analysis. The on-line extraction of Dextrorphan / Dextromethorphan plasma samples was used for the cross validation method test.

RTC sample preparation method

Plasma samples (100 µL) were put in injection vials, capped then transferred to the RTC system. The on-line protein precipitation was performed as follows: automated addition of 800 µL acetonitrile solution containing internal standard in sample vial using 1 mL syringe and vial was transferred to a vortex mixer. During the phase separation, RTC syringe tool automatically changes from a 1 mL to a 10 µL and acetonitrile phase is injected in LC-MS/MS system followed by LazWell plate spotting. LC-MS/MS and LDTD-MS/MS analysis were performed on a Sciex QTrap 5500.

LDTD-APCI-MS/MS method

For the LDTD-MS/MS analysis, following the on-line extraction, 5 µL of protein precipitation sample was added in LazWell plate and evaporated to dryness. Samples were desorbed using the following laser pattern: increase laser power to 25% in 6 seconds, maintain 2 seconds at 25% laser power and decrease laser power to 0% in 0.1 second. An air flow of 3 L/min is used as a carrier gas. Positive APCI ionization mode with 3 µA discharge current was used with 258®199 and 272®215 as transitions for Dextrorphan and Dextromethorphan respectively.

LC-MS/MS method

After the on-line extraction, 5 µL of protein precipitation sample was injected on a SB-C18 column (4.6 X 200, 5µm) for LC-MS/MS analysis. A flow rate of 1 mL/min (isocratic mode) using Acetonitrile:Water (50:50) with 0.1% FA as mobile phase was used. Drugs were detected in positive ESI mode using 258®157 and 272®215 as transition for Dextrorphan (DEX) and Dextromethorphan (DMX) respectively. The retention time of Dextrorphan and Dextromethorphan were 3.2 and 4.5 minutes respectively.

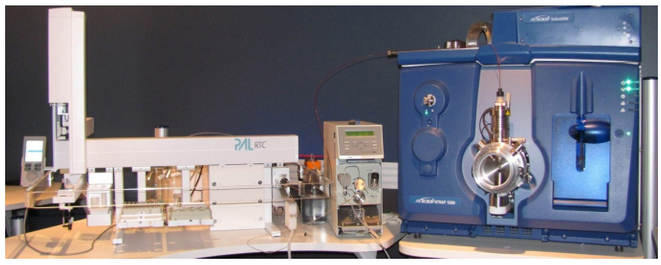

Fig. 1: RTC system and a LC coupled to a MS/MS Sciex QTRAP® 5500 System in MRM mode

Results and Discussion

LC-MS/MS

Linearity of 0.99638 (DEX) and 0.99842 (DMX) with a calibration range of 0.5 to 100 ng/mL is obtained (Figures 2 & 3). Intra-run accuracy is between 93 and 115 % and precision is below 11 % (Table 1).

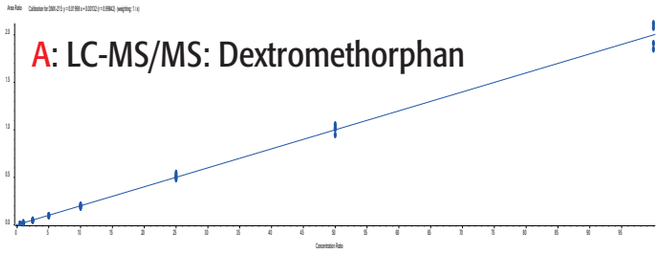

Fig. 2: DMX calibration curve with LC-MS/MS

Fig. 3: DEX calibration curve with LC-MS/MS

Compound	Conc (ng/ML)	n	Mean (ng/mL)	%RSD	%NOM
DEX	0.5	4	0.57	6.22	114.76
	2.5		2.33	6.43	93.14
	10		9.72	5.21	97.20
	50		50.33	6.52	100.67
	100		100.80	9.45	100.80
DMX	0.5		0.53	10.36	106.39
	2.5		2.39	8.41	95.78
	10		9.82	7.83	98.17
	50		50.43	4.53	100.86
	100		99.48	6.50	99.48

Tab. 1: DEX and DMX intra run accuracy with LC-MS/MS

LDTD-MS/MS

Linearity of 0.99785 (DEX) and 0.99791 (DMX) with a calibration range of 0.5 to 100 ng/mL is obtained (Figures 4 & 5). Intra-run accuracy is between 80 and 107 % and precision is below 10% (Table 2).

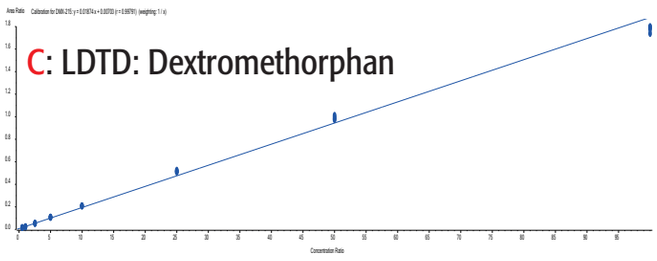

Fig. 4: DMX calibration curve with LDTD-MS/MS

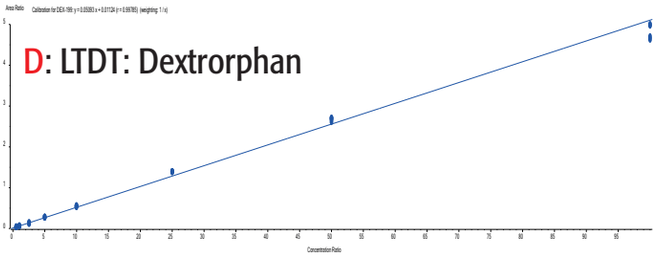

Fig. 5: DEX calibration curve with LDTD-MS/MS

Cross validation

Cross validation of unknown samples was performed (Figures 6 & 7). A correlation of 0.9987 and 0.9981 is obtained between LC-MS/MS and LDTD-MS/MS results for Dextrorphan and Dextromethorphan, respectively.

Compound	Conc (ng/ML)	n	Mean (ng/mL)	%RSD	%NOM
DEX	0.5	4	0.43	8.34	86.13
	2.5		2.48	1.13	99.24
	10		10.61	1.85	106.06
	50		52.40	1.39	104.81
	100		94.70	3.87	94.70
DMX	0.5		0.41	8.94	81.08
	2.5		2.54	3.95	101.47
	10		10.69	1.10	106.87
	50		52.44	1.71	104.88
	100		94.48	1.67	94.48

Tab. 2: DEX and DMX intra run accuracy with LDTD-MS/MS

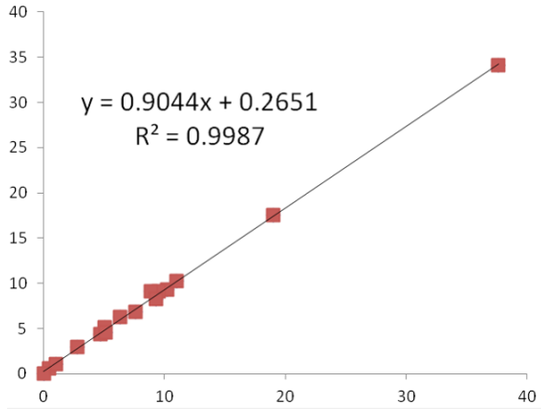

Fig. 6: Cross-validation between LC and LDTD (Dextrorphan)

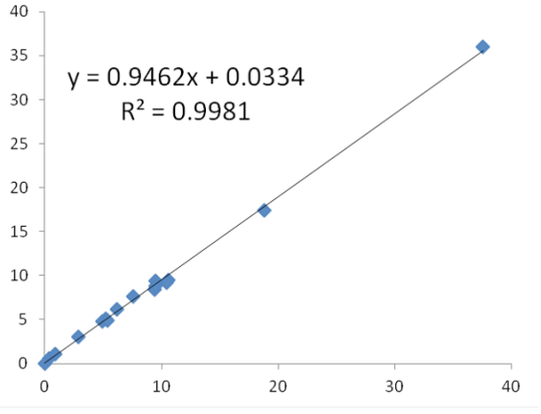

Fig. 7: Cross-validation between LC and LDTD (Dextromethorphan)

Conclusions

The Robotic Tool Change (RTC) effectively performs on-line automated protein precipitation for the analysis of Dextromethorphan and Dextrorphan. The extracts were analysed using 2 techniques: LC-MS/MS and LDTD-MS/MS. The RTC is an excellent tool for a cross-validation between two different techniques.

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