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Application News

Fully automated sample preparation module: CLAM™-2030
High Performance Liquid Chromatograph Triple Quadrupole Mass Spectrometer: LCMS™-8045/8050/8060/8060NX

Fully automated quantification of Meropenem, Tazobactam, Piperacillin and Dexamethasone in plasma

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User Benefits

- Simultaneous quantification of a corticosteroid and 3 antibiotics
- Rapid analysis within 5 min and fully automated sample preparation
- Sufficient repeatability and robustness

■ Introduction

This application describes the analysis of one corticosteroid Dexamethasone and 3 antibiotics Tazobactam, Meropenem and Piperacillin. Dexamethasone is a synthetic glucocorticoid hormone. It has an anti-inflammatory and immunosuppressive effect. It is widely used for reducing swelling associated with tumors, and treat eye inflammation. Dexamethasone can help treat allergic reactions and be used in cancer treatments too[1].

More recently, studies have demonstrated the effectiveness of dexamethasone in the treatment of COVID-19, mainly for people with acute respiratory distress syndrome (ARDS). Corticosteroid therapy reduced the risk of all-cause mortality (risk ratio 0.75; 95% CI, 0.59–0.95)[2].

In order to avoid the risk of bacterial overgrowth, which is more common with corticosteroid treatment, an antibiotic therapy is often combined with it[2].

The following method allows the simultaneous quantification of corticoid and antibiotic in biological samples with a fully automated sample preparation due to the use of CLAM system.

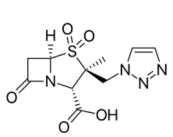
By simplifying uncapping blood collection tubes (or sample cups), placing the pretreatment vials in the system, and requesting analysis, the system performs all other process steps automatically, from pretreatment to LCMS analysis.

This fast analytical method (=5min) and its automatic and overlapped sample preparation allows to treat a large number of samples in a minimum of time. The sensitivity of the LCMS-8060 allows to sweep samples with an analysis dynamic range from 1 to 100 ng/mL.

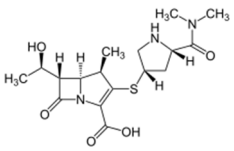
■ Method

This application describes the analysis of one corticosteroid Dexamethasone and 3 antibiotics (Fig.1). Tazobactam, Meropenem and Piperacillin with a dynamic range of quantification from 1 to 100 ng/mL. The standard powders of each compound and of the internal standard [$^2H_4$]-Dex amethasone was purchased from Alsachim (Strasbourg, France). The solvent used were provided from FUJIFILM Wako Pure Chemical and ammonium fluoride was from Sigma-Aldrich. The analytical system consisted of a Shimadzu Nex era™ X2, a LCMS-8060 with a CLAM-2030 in front (Fig.2). The MRM transitions were optimized by using flow injection analysis (FIA) for all compounds. The source parameters were optimized to improve the ionization and desolvation of these compounds and consequently to increase their sensitivity. The method was developed on plasma.

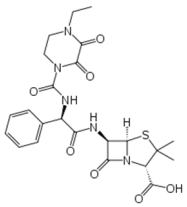
The optimized analytical conditions used in liquid chromatography and the mass spectrometry are shown in Table 1 and 2, respectively.
The sample preparation was processed by the CLAM-2030. The plasma, all solvents and acid used are purchased from Wako Pure Chemical. The solution of internal standard has a concentration of 10 ng/mL. The different step are described in the figure 3.



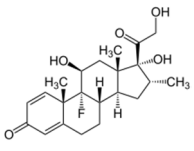
Tazobactam
(P/N: C4573*)



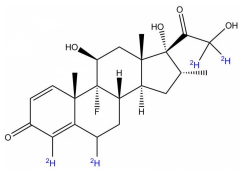
Meropenem
(P/N:C3413*)



Piperacillin
(P/N:C3387*)



Dexamethasone
(P/N:C5057*)



[$^2H_4$]-Dexamethasone
(P/N:C4923*)

Figure 1 **Structure of each compounds**

*Alsachim Product Number

Figure 2 **Fully automated LC/MS/MS system**

Table1 **Liquid Chromatography Conditions**

Liquid chromatograph		
System	:	Nexera X2
Column	:	Shim-pack Scepter™ C18-120 (50 mm × 2.1 mm I.D., 1.9µm)
Temperature	:	30 °C
Injection volume	:	25 µL
Mobile phases	:	0.1mM ammonium fluoride-Water 0.1mM ammonium fluoride-Methanol
Flow rate	:	600 µL/min
Analytical time	:	5 min

Table2 **Mass Spectrometry Conditions**

Mass spectrometer		
System	:	LCMS-8060
Nebulizing gas	:	3 L/min
Drying gas	:	10 L/min
Heating gas	:	10 L/min
DL temp	:	150 °C
Heat block temp	:	500 °C
Interface temp	:	400 °C
CID	:	270 kPa

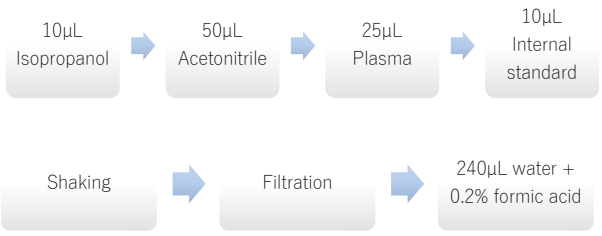


Figure 3 **Sample preparation**

■ Results

Calibration data and limit of quantification

The calibration curve obtained have a dynamic range from 0.4 ng/mL to 100 ng/mL. The calibration point are prepared in plasma from standards solution at 0.4, 1, 2, 5, 10, 25, 50 and 100 ng/mL. The curve obtained are linear with a coefficient of determination greater than 0.995. The limit of quantification are fixed for all compounds at 1 ng/mL. The accuracies obtained are between 85-115%. The calibration samples diluted with water are presented in figure 4.

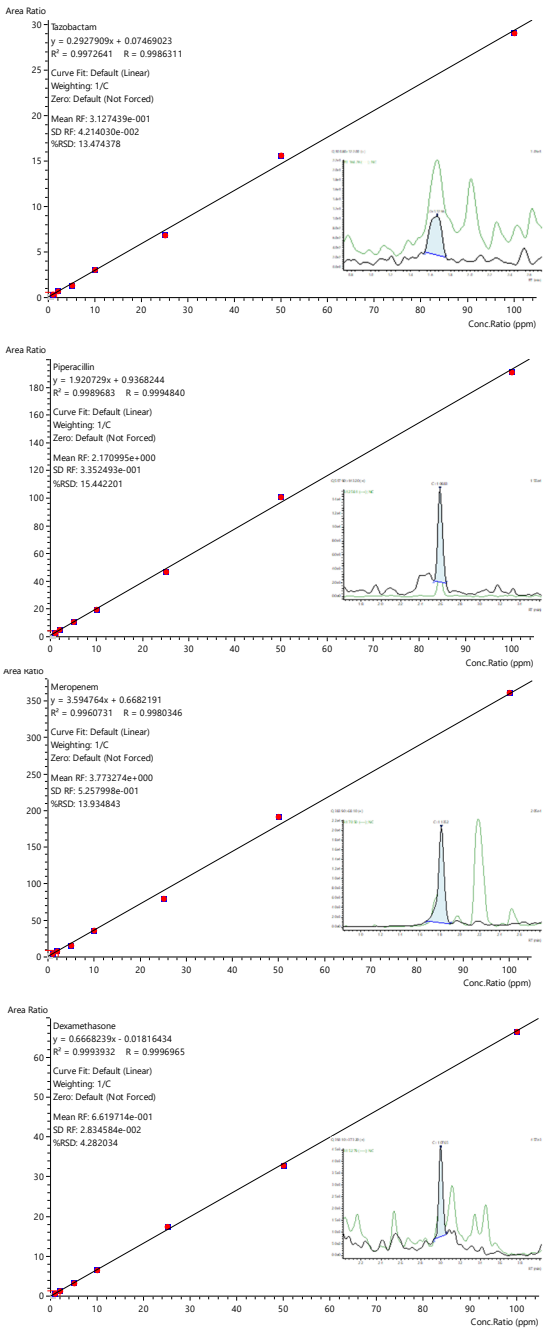


Figure 4 **Calibration curve and chromatogram for Tazobactam, Piperacillin, Meropenem and Dexamethasone at the limit of quantification 1 ng/mL**

Table 3 **Between-days reproducibility and accuracies of each drug**

Compounds	QC Sample	Spiked Conc. (ng/mL)	Day 1st (*n*=6)			Day 2nd (*n*=3)			Day 3rd (*n*=3)		
			Average Conc. (ng/mL)	Precision %RSD	Accuracy %	Average Conc. (ng/mL)	Precision %RSD	Accuracy %	Average Conc. (ng/mL)	Precision %RSD	Accuracy %
Tazobactam	Low	2	2.22	11.8	111	2.14	7.44	107	1.74	12.5	87
	Medium	20	22.6	6.12	113	18.9	9.60	94	18.5	3.04	92
	High	80	86.4	7.56	108	73.4	8.28	92	71.3	3.82	89
Piperacillin	Low	2	2.05	7.53	103	2.13	14.2	107	1.81	6.38	91
	Medium	20	20.9	11.9	104	20.1	14.5	101	21.5	5.41	107
	High	80	76.8	10.8	96	77.4	5.86	97	81.4	3.82	102
Meropenem	Low	2	2.00	5.86	100	2.12	12.0	106	1.788	2.59	89
	Medium	20	22.1	8.29	111	18.6	11.4	93	18.4	3.44	92
	High	80	80.2	9.01	100	73.3	2.61	92	72.6	3.45	91
Dexamethasone	Low	2	1.98	11.5	99	1.98	14.9	99	1.82	8.01	91
	Medium	20	20.8	9.57	104	22.6	14.1	113	20.4	6.88	102
	High	80	82.6	12.0	103	79.6	7.86	99	83.7	4.90	105

Accuracy and repeatability

In order to assess repeatability and accuracy of the methods, 6 controls were prepared for Day 1st and 3 controls were prepared for Day 2nd and Day 3rd, respectively. 3 levels of control were used: low at 2 ng/mL, medium at 20 ng/mL and high at 80 ng/mL. Controls are prepared by spiking standard solutions in plasma. After a minimum wait of 1 hour, the controls are prepared using CLAM-2030. The table 3 present the repeatability and accuracy obtained for each series of controls on each level. The precision of Tazobactam (%RSD) was 12.5% or below and the accuracy was ranged between 87% – 113%. The precision of Piperacillin (%RSD) was 14.5% or below and the accuracy was ranged between 91% – 107%. The precision of Meropenem (%RSD) was 12.0% or below and the accuracy was ranged between 89% – 111%. The precision of Dexamethasone (%RSD) was 14.9% or below and the accuracy was ranged between 91% – 113%. All reproducibility were less than 15% and accuracies were between 85-115%.

■ Summary and Conclusion

The Shimadzu LCMS-8060 and the CLAM-2030 allows the simultaneous quantification of the corticosteroid Dexamethasone and 3 antibiotics Tazobactam, Meropenem and Piperacillin. This method allows a rapid analysis with a 5 min run and a fully automated sample preparation. The sensitivity of the LCMS-8060 allows to sweep the samples with an quantitative dynamic range from 1 to 100 ng/ml. The good repeatability less than 15% and the accuracy between 85-115% on each control sample confirmed the robustness and the efficiency of this method.

■ Reference

1) https://www.antibioticresearch.org.uk/dexamethasone-evidence-based-treatment-for-hospitalised-covid-19-patients/
2) https://www.covid19treatmentguidelines.nih.gov/immune-based-therapy/immunomodulators/corticosteroids/

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