thermo scientific

SIMULTANEOUS DETERMINATION OF CLIDINIUM & CHLORDIAZEPOXIDE ON LCMSMS USING CLIDINIUM-D3 & CHLORDIAZEPOXIDE-D5 AS INTERNAL STANDARD & DATA ANALYSIS THROUGH CHROMELEON 7.2.10 CDS SOFTWARE SOLUTION

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ABSTRACT

Purpose : To represent an assay performance utilizing TSQ-Altis highly sensitive MS system for the simultaneous quantitation of Clidinium and Chlordiazepoxide in Human plasma using Clidinium-d3 and Chlordiazepoxide-d5 as internal standards, respectively.

Methods : Clidinium and Chlordiazepoxide were analyzed in the presence of human plasma sample matrix using Selected Reaction Monitoring in TSQ Altis Mass spectrometer and data analysis was done by Thermo Scientific Chromeleon 7.2.10 software.

Results : The lower limit of quantitation (LLOQ) for Clidinium and Chlordiazepoxide was determined to be 10 pg/mL and 1ng/mL, respectively.

INTRODUCTION

Chlordiazepoxide/clidinium bromide (marketed as Librax) is a drug consisting of chlordiazepoxide (marketed separately under the trade name Librium) and clidinium bromide and used to treat peptic ulcers, irritable bowel syndrome (IBS), and gastritis. It helps relieve stomach spasms, abdominal cramps, and anxiety related to gastric disorders. Librax is a fixed ratio of these two medications and, as such, is not typically prescribed with an accompanying dosage, but rather directions on how many capsules to take per day. It comes in a capsule taken by mouth, usually three or four times daily, before meals and at bedtime. Chlordiazepoxide is an anti-anxiety medication belonging to the benzodiazepine class. Its use in IBS is thought to be due to its calming ability for patients that have IBS symptoms that are worsened by anxiety. Clidinium bromide is a synthetic quaternary ammonium antimuscarinic, a sub-class of a family of drugs known as anticholinergics. It treats IBS by decreasing gastrointestinal motility.

For this combination of a single dose of Chlordiazepoxide(5mg)/clidinium bromide(2.5mg), although the exact figures representing the peak plasma concentration are not available in literature, still as per the most relevant information, the Cmax is approximately 200ng/mL/1200pg/mL, respectively and thus there is a requirement of selective and sensitive quantitative analysis with linear dynamics of LCMS/MS instrument to support both nanogram and picogram levels together in the same run & with capability to produce reproducible and reliable concentration data for pharmacokinetic analysis and support in development of better formulations in future.

Here we evaluated the advantage of highly sensitive, selective and robust TSQ Altis mass spectrometer in achieving the accurate and precise concentrations of Chlordiazepoxide and Clidinium in human plasma. A rational approach was investigated to evaluate the robustness of the instrument and overall assay performance of this LC-MS/MS analysis. The main challenges observed were interferences especially with Clidinium by keeping high volume of plasma as well as addition to the back pressures which was eliminated by reducing plasma volume that in turn reduced matrix factor significantly.

MATERIALS AND METHODS

Sample Preparation

Clidinium Bromide and Chlordiazepoxide HCl working standards were dissolved in appropriate solvent to achieve a concentration of 1mg/mL. This standard was used to prepare calibration curve and Quality control (QC) dilutions. These dilutions were prepared in diluent solution of the method that after spiking into plasma should make a suitable concentration range starting from 1 ng/mL and 10 pg/mL for Chlordiazepoxide HCl and Clidinium Bromide respectively. Human plasma was filtered and used to generate Calibration curves and QC samples.

Spiked Calibration curve standards and quality control samples were prepared after adding appropriate volumes of Chlordiazepoxide HCl and Clidinium Bromide dilutions in human plasma at each calibration curve and quality control concentrations. Only 150 µL aliquot from each spiked sample was taken for sample processing which was finally reconstituted with 1000 µL of Reconstitution solution. 5 µL of the samples was injected for analysis on LC-MS/MS system.

Liquid Chromatography

Chromatographic separation was achieved using a Thermo Scientific Vanquish UHPLC system. Samples were injected at 5 µL. Gradient elution was accomplished with a total runtime of 5 minutes.

Mass Spectrometry

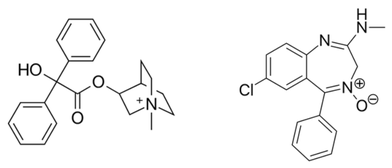
Chlordiazepoxide and Clidinium were analyzed using Thermo Scientific TSQ Altis MS with Heated Electrospray Ionization (Optamax NG Ion Source). Appropriate source conditions suitable for desolvating the complete LC flow and for good sensitivity of both the analytes were applied for all data collection. Data were acquired in SRM mode for Chlordiazepoxide & Clidinium using Chlordiazepoxide-d5 and Clidinium-d3 as internal standards, respectively.

Data Analysis

All the data were acquired and processed utilizing Thermo Scientific Chromeleon 7.2.10 Software. All chromatographic integrations were accomplished using method defined processing settings. No manual integration was applied to any chromatographic data.

RESULTS

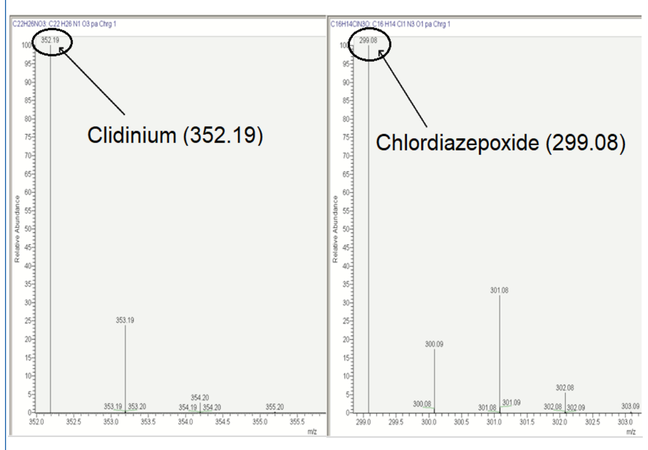
Figure 1. Chemical structure & Formula of Clidinium & Chlordiazepoxide



Clidinium (C22H26NO3+)

Chlordiazepoxide (C16H14ClN3O)

Figure 2. Exact mass of Clidinium & Chlordiazepoxide



The LC-MS/MS analysis of Clidinium and Chlordiazepoxide was performed on a Vanquish UHPLC system coupled with TSQ Altis MS in SRM mode. This hyphenated system is easy to handle and enables highly selective and sensitive determination of compounds from complex sample matrices.

Clidinium and Chlordiazepoxide were tuned using exact mass (as in Figure 2) given by Thermo Scientific Xcalibur Qual Browser. After Tune & Optimization, the final SRM transition as well as best suitable ion source parameters were selected for this analysis.

Figure 3. Chromatograms of Clidinium in Human Plasma Samples - Blank, LLOQ (10.0 pg/mL) and ULOQ (2500.0 pg/mL)

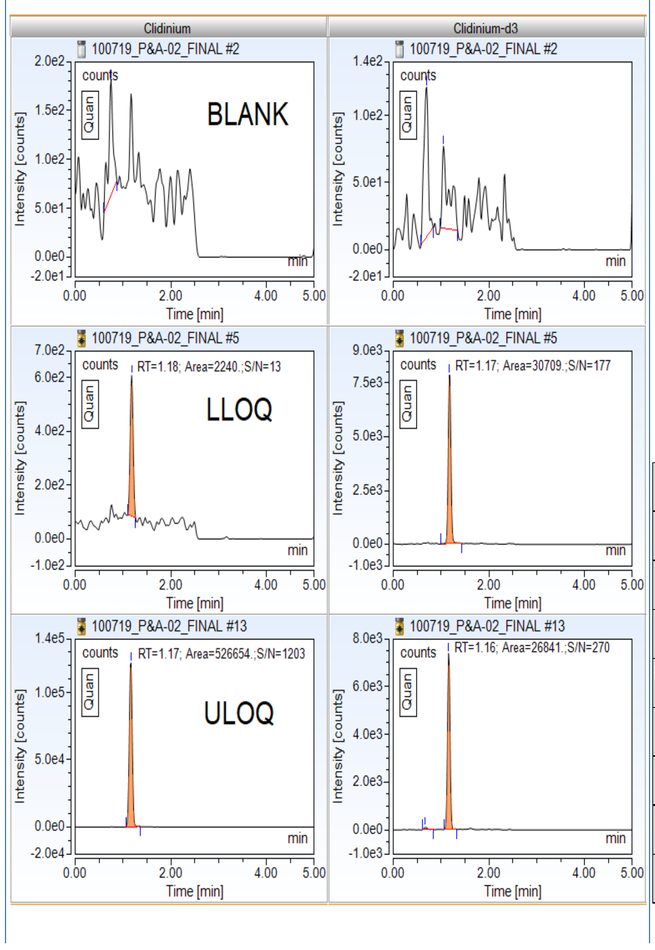
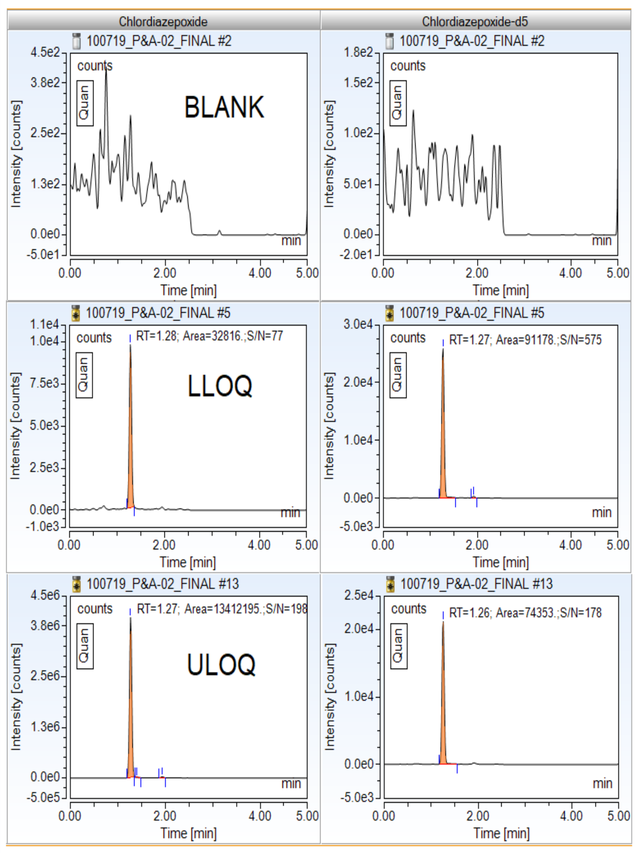


Figure 4. Chromatograms of Chlordiazepoxide in Human Plasma Samples - Blank, LLOQ (1.00 ng/mL) and ULOQ (500.00 ng/mL)



Chromatographic separation was performed to clear maximum interfering peaks and achieved the final chromatography as in Figure 3 and Figure 4 for Extracted Plasma Blank, LLOQ (Lower limit of quantification) and ULOQ (Upper limit of quantification) for both the analytes. This LC-MS method was found best to achieve good sensitivity, selectivity, reproducibility and robustness with improved column cleanup within each sample run.

Tuning was performed with a mix solution of 500 ng/mL concentration of both Clidinium & Chlordiazepoxide that gave a clear identification and intensity of Precursor ion for both compounds and with same solution, very stable and intense product ions were also obtained.

SRM Analysis

LC gradient setup in this analysis benefits in good separation of interferences from the RT of both the analytes that was achieved during development phase of this method where the effect of matrix from one sample to another in long run was found to be nil. During this study the main focus was targeted as Good precision and accuracy, no matrix factor and good extraction recovery.

The extraction recovery of the method was found to be **≥70%** at all the low, middle and high quality control concentrations for both Clidinium and Chlordiazepoxide.

Chromatographic results, as shown in Figure 3 & Figure 4, were found stable in terms of RT, Response and Reproducibility. Further, the results present in Table 1 & Table 2 show the precise and accurate quantitation and no matrix factor. Lower limit of quantitation gives excellent S/N ratio, shown in peak label in Figure 3 & Figure 4, and further explains the sensitivity of the Mass spectrometer and excellent performance of Vanquish UHPLC at low flow rate.

Assay Performance

Assay performance was determined from validation experiments like Precision and accuracy batch, Matrix factor & Recovery evaluation and response stability in long run and was found to be fit for purpose. The results are tabulated and presented here in Table 1 & Table 2. The Calibration curves are shown in Figure 5.

Since, there is high level of variability in pharmacokinetics from person to person, ethical and regulatory norms are there with certain criteria and specific ways of performing validation and clinical studies with proper guidance therefore, all the criteria must be met.

The values obtained as a result of the experiments performed in this analysis are well within all acceptance limits of regulatory guidelines.

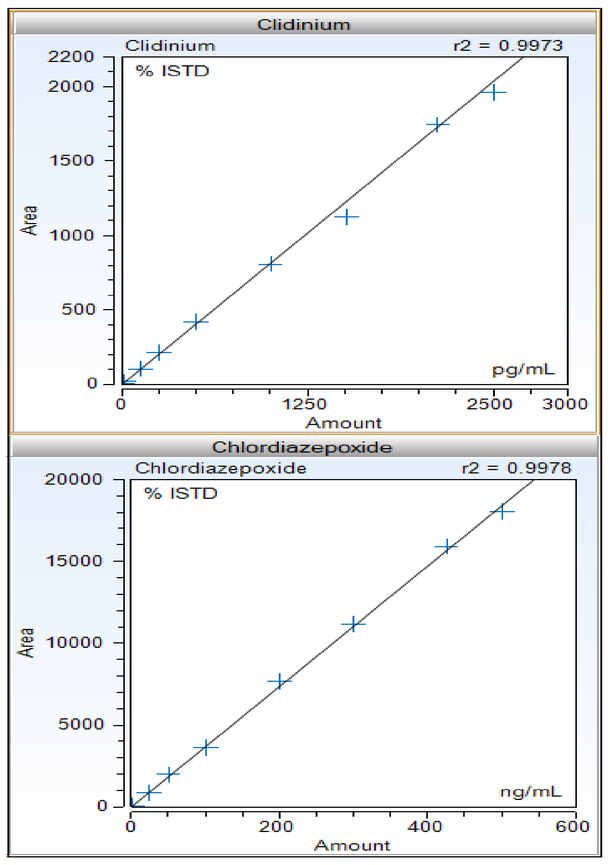
Table 1. Clidinium - Assay performance results

Clidinium					
Precision & Accuracy (6 replicates)					
QC	LOQQC	LQC	M1QC	MQC	HQC
Concentration (pg/mL)	10.5	29.9	180.0	1250.0	1760.6
Mean Accuracy (%)	115.0	101.0	96.2	101.1	100.7
Precision (%)	15.8	4.3	2.4	2.2	1.7
Matrix Factor					
QC	LQC	-	HQC		
Matrix Factor (%CV)	4.8	-	2.2		

Table 2. Chlordiazepoxide - Assay performance results

Chlordiazepoxide					
Precision & Accuracy (6 replicates)					
QC	LOQQC	LQC	M1QC	MQC	HQC
Concentration (pg/mL)	1.0	3.0	40.6	250.3	376.4
Mean Accuracy (%)	109.9	89.5	96.7	97.4	97.7
Precision (%)	2.0	4.1	2.6	1.7	2.3
Matrix Factor					
QC	LQC	-	HQC		
Matrix Factor (%CV)	2.2	-	1.3		

Figure 5. Calibration curve



The overall assay performance proves this excellent combination of TSQ-Altis with Vanquish UHPLC to be a fit for purpose LCMSMS system and determines the Lower limit of quantification as 10 pg/mL for Clidinium and 1 ng/mL for Chlordiazepoxide. This concentration has been achieved by taking only 150µL plasma volume, reconstituting it with 1000µL and injecting only 5µL which further proves the high sensitivity of the system that allows to take minimum plasma volume and inject low sample volumes onto the column thereby enhancing system's robustness.

CONCLUSIONS

SRM analysis on TSQ Altis MS provides confident quantitation. The LLOQ for Clidinium & Chlordiazepoxide was determined to be 10 pg/mL and 1 ng/mL, respectively and was found reproducible and appropriate for a quantitative analysis with linear response over a range of approx. 10 to 2500 pg/mL & 1 to 500 ng/mL for Clidinium and Chlordiazepoxide, respectively.

The assay performance was good and acceptable from the regulatory point of view as well as the ethical requirement of using low sample volume for analysis.

This system configuration viz. Vanquish UHPLC coupled with TSQ Altis MS proves here its robustness and very high level of assay selectivity with complete removal of matrix interferences even at low flow rates.

Detector sensitivity and SRM method selectivity coupled with an efficient and reproducible Solid phase extraction provides rugged and appropriate assay for the selective & simultaneous quantitative analysis of Clidinium & Chlordiazepoxide with Clidinium-d3 & Chlordiazepoxide-d5 as internal standards, respectively.

REFERENCES

1. Simultaneous Determination of Clidinium Bromide and Chlordiazepoxide in Combined Dosage Forms by High-Performance Liquid Chromatography, Safwan Ashour and Nuha Kattan, Analytical Biochemistry Laboratory, Department of Chemistry, Faculty of Science, University of Aleppo, Aleppo, Syria

TRADEMARKS/LICENSING

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