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Note d'application

Benefits of Using QDa Mass Detection for Quantitative Analysis of Non-Chromophoric Memantine HCl in Tablet Formulation

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Abstract

This application note describes the use of the ACQUITY QDa module, a small but robust, simple-to-use mass detector, to quantitate non-chromophoric memantine HCl, a drug commonly used to treat dementia often associated with Alzheimer's disease.

Benefits

- Easy and direct technique for analyzing non-chromophoric compounds with the ACQUITY QDa Detector
- Accurate identification of components by mass detection with the ACQUITY QDa Detector
- Eliminate the need for complex, time-consuming derivatization procedures
- Compatibility with existing UPLC systems and methodologies

Introduction

The analysis of compounds that lack UV chromophores or that have low UV-extinction coefficients can be challenging. Because these compounds cannot be directly detected by UV, their identification and measurement must depend on alternate methods. For all pharmaceutical products, it is particularly important to correctly identify its components, for failing to do so can compromise the drug's safety, efficacy, or both.

For components that exhibit poor UV absorbance or none, samples often require pre-or post-column derivatization, for UV detection. In the case of memantine hydrochloride, a non-chromophoric drug compound, several methods are found in the literature for quantitative determination in dosage. Few methods for determining memantine HCl in drug formulations have been reported, including HPLC methods with UV-detection by means of a pre-column derivatization technique[1,2] and GC-FID methods.[3,4] While effective and sensitive, these methods are not ideal for routine testing in a quality control laboratory. They require tedious and complex pre-column derivatization procedures or additional instrumental analysis such as gas chromatography. The lack of straightforward methods can lead to additional undesirable variability in response. This often requires additional method-development time, robustness testing, user training, and monitoring in order to improve the quality of the assay. Mass detection, on the other hand, enables quick and accurate determination of non-chromophoric compounds, and can eliminate the need for complex sample-preparation procedures.

In this application note, we describe the use of the ACQUITY QDa module, a small but robust, simple-to-use mass detector, to quantitate non-chromophoric memantine HCl, a drug commonly used to treat dementia often associated with Alzheimer's disease. Here, we present a UPLC method coupled with the ACQUITY QDa Detector to quantitatively determine memantine HCl in the drug tablet formulation. We will demonstrate the system suitability, method linearity, and specificity achievable with mass detection for routine assays.

Overall, mass detection provides quick identification and analysis of non-chromophoric compounds, accurate and reliable results. Thus, mass detection is complementary to UV techniques and suitable for routine testing in the QC laboratory.

Experimental

UPLC method conditions

LC system:	ACQUITY UPLC H-Class

Column:	ACQUITY UPLC CORTECS C_{18+}, 2.1 x 50 mm, 1.6 µm
Column temp.:	45 °C
Flow rate:	0.6 mL/min
Injection volume:	1.0 µL
Solvent A:	125 mM Formic acid in water
Solvent B:	Water
Solvent C:	Acetonitrile
Separation:	Gradient

Step	Time(min)	Solvent A(%)	Solvent B(%)	Solvent C(%)	Curve
1	Initial	10.0	85.0	5.0	Initial
2	2.5	10.0	42.5	47.5	6
3	2.6	10.0	0.0	90.0	6
4	3.1	10.0	0.0	90.0	6
5	3.2	10.0	85.0	5.0	6
6	5.0	10.0	85.0	5.0	6

Purge/Sample wash:	50:50 water/methanol

Seal wash:	90:10 water/acetonitrile
UV detector:	ACQUITY UPLC PDA: 210–400 nm (derived at 210 nm)

MS conditions

Mass detector:	ACQUITY QDa (Extended Performance)
Ionization mode:	ESI+
MS Acquisition range:	100 – 300 Da
Single Ion Recording:	180.2 Da
Sampling rate:	10 pts/s
Capillary voltage:	0.8 kV
Cone voltage:	15 V
Probe temperature:	600 °C
Data:	Centroid
System Control, Data Acquisition, and Analysis:	Empower 3 FR2 CDS Software

Solutions preparation

Standard solutions

The memantine HCl stock solution was prepared in methanol at a concentration of 1.0 mg/mL. The stock solution was then diluted with standard diluent (10:90 methanol/water) to a working concentration of 0.005

mg/mL. The working standard solution was used to prepared linearity standards by dilution with standard diluent (10:90 methanol/water). Linearity standards were prepared at these concentrations: 0.05, 0.10, 0.15, 0.25, 0.50, 0.75, and 1.00 µg/mL.

Tablet sample solutions

Stock sample solutions were prepared by dissolving tablets containing 10 mg of memantine HCl in 50:50 0.1 N HCl/ethanol,* to a concentration of 1.0 mg/mL. The solutions were sonicated and centrifuged, at 3500 rpm, for 30 minutes. Finally, the solutions were filtered through 0.2-µm GHP membrane syringe filters* and diluted with sample diluent (0.1N HCl) to the working concentration of 0.75 µg/mL.

**Final method, other dissolution and filtering conditions tested and presented in this application note.*

Results and Discussion

Memantine HCl is a tricyclic amine that lacks the chromophores required for UV detection (Figure 1). Thus it cannot be directly detected by UV. Nevertheless, it is readily ionizable, producing a robust MS signal on the ACQUITY QDa Detector. Figure 2 shows several detector traces. As expected, the UV trace at 210 nm for memantine at 1 µg/mL shows no discernible peak. (Figure 2a.)

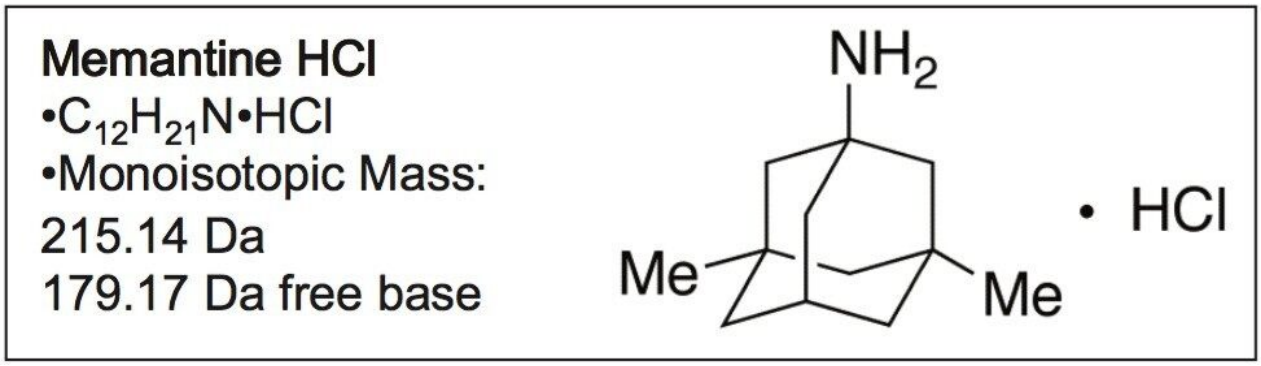


Figure 1. Chemical information and structure of Memantine HCl.

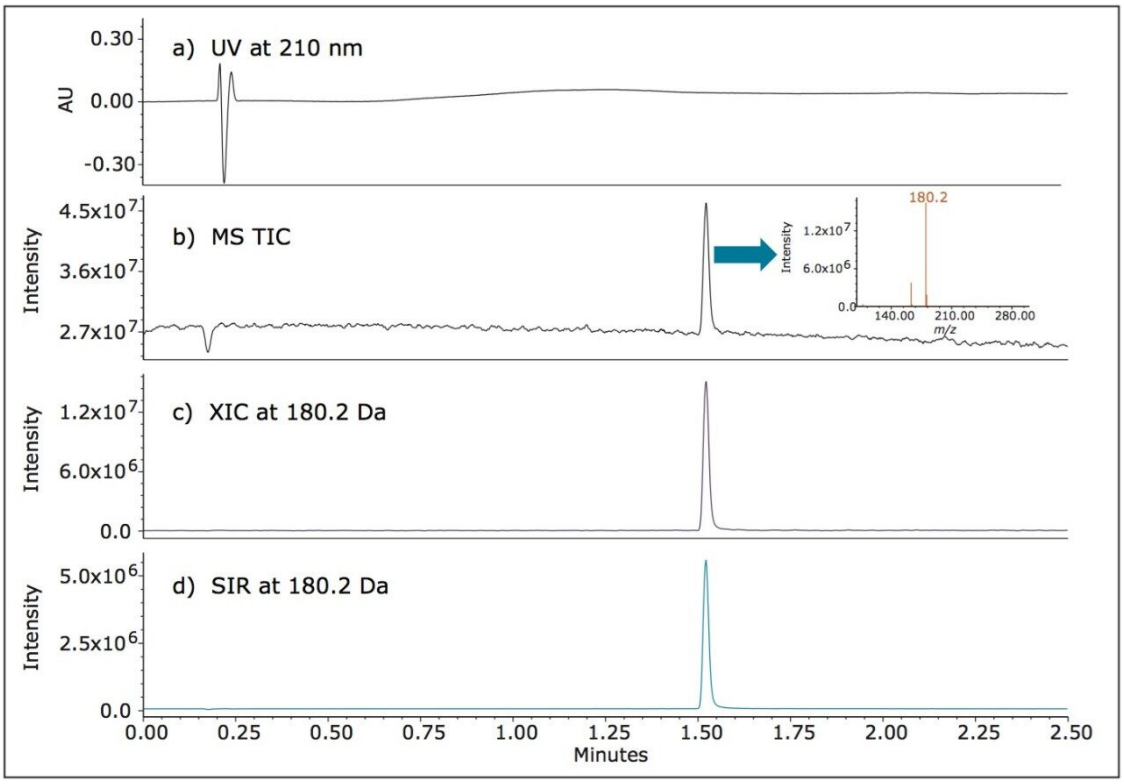


Figure 2. UV and MS chromatographic data for memantine HCl acquired using the ACQUITY UPLC H-Class System with PDA and ACQUITY QDa Detector.

System Suitability

Performance of the UPLC method was verified by evaluating repeatability of five replicate injections of the 1 µg/mL standard (Figure 3) made according to the specifications defined in the USP General Chapter, <621> "Chromatography".[5] The UPLC system suitability results, processed using SIR mass data at 180.2 Da, are shown in Figure 4. The retention times and area repeatability were well within the USP specification of less than 2% RSD.

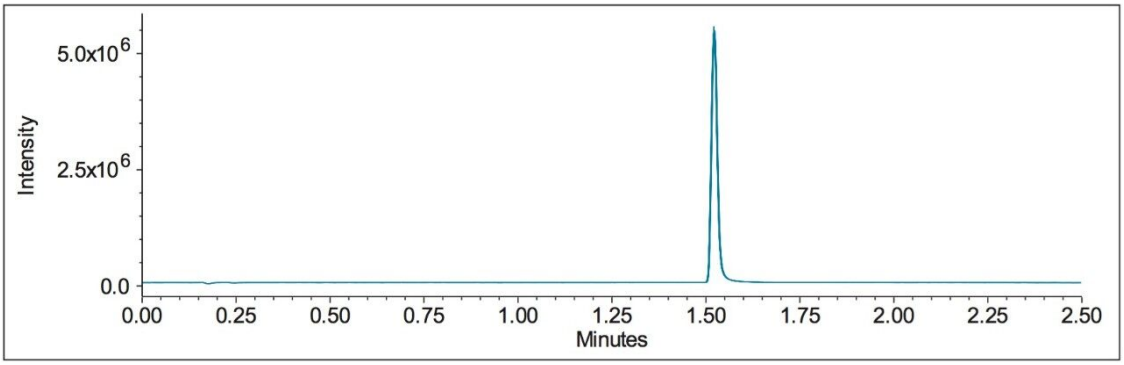


Figure 3. Overlay of five replicate injections of Memantine HCl standard: SIR mass data at 180.2 Da.

System Suitability_Report

Sample Set ID: 12773
Result Set ID: 14455
Channel Name: QDa 3: SIR Ch1

Name: Memantine

	Name	SampleName	Inj	RT	Area	USP Tailing	K Prime
1	Memantine	Memantine SS: 1ug/mL	1	1.523	6071507	1.2	5.1
2	Memantine	Memantine SS: 1ug/mL	2	1.522	6076992	1.2	5.1
3	Memantine	Memantine SS: 1ug/mL	3	1.521	6107702	1.2	5.1
4	Memantine	Memantine SS: 1ug/mL	4	1.521	6139821	1.2	5.1
5	Memantine	Memantine SS: 1ug/mL	5	1.521	6157180	1.2	5.1
Mean				1.522	6110640	1.2	5.1
Std. Dev.				0.001	37714.946		
% RSD				0.06	0.62		

Figure 4. System suitability results for five replicate injections of standard solution: SIR mass data at 180.2 Da.

Linearity

Linearity of the method for memantine HCl with mass detection was evaluated over seven concentrations

ranging from 0.05 µg/mL to 1.0 µg/mL. The method showed good linear correlation between the peak areas and concentrations of memantine HCl, with correlation coefficients (R^2) ≥0.998 (Figure 5). In addition, the percent deviation of the calculated x values or concentrations were less than 7.0% (Figure 6).

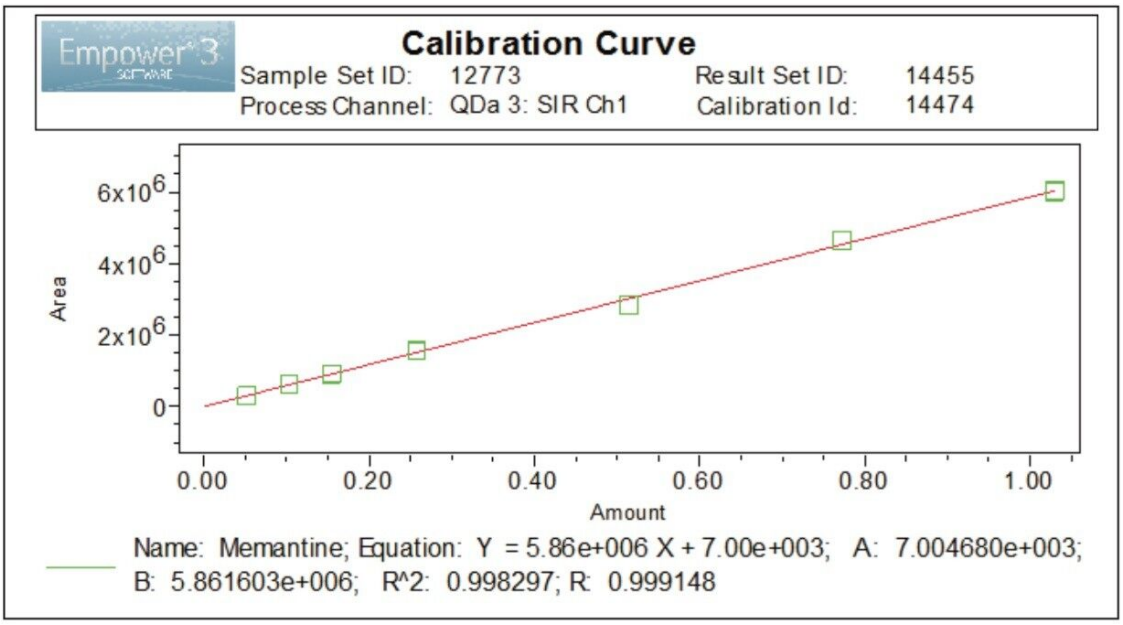


Figure 5. Linearity of the method for memantine HCl. Data processed using SIRmass data at 180.2 Da.

Calibration Curve %Deviation

Sample Set ID: 12773 Result Set ID: 14455

Channel Name: QDa 3: SIR Ch1

Peak MemantineX Value (ug/mL): 0.05150

	Name	Level	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	Memantine	1	1	305873	0.05150	0.05099	-0.995
2	Memantine	1	2	309364	0.05150	0.05158	0.161
3	Memantine	1	3	305437	0.05150	0.05091	-1.139

Peak MemantineX Value (ug/mL): 0.10300

	Name	Level	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	Memantine	2	1	635819	0.10300	0.10728	4.152
2	Memantine	2	2	625800	0.10300	0.10557	2.493
3	Memantine	2	3	639816	0.10300	0.10796	4.814

Peak MemantineX Value (ug/mL): 0.15450

	Name	Level	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	Memantine	3	1	920154	0.15450	0.15578	0.832
2	Memantine	3	2	917046	0.15450	0.15525	0.488
3	Memantine	3	3	911865	0.15450	0.15437	-0.084

Peak MemantineX Value (ug/mL): 0.25750

	Name	Level	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	Memantine	4	1	1561034	0.25750	0.26512	2.959
2	Memantine	4	2	1566506	0.25750	0.26605	3.322
3	Memantine	4	3	1579787	0.25750	0.26832	4.202

Peak MemantineX Value (ug/mL): 0.51500

	Name	Level	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	Memantine	5	1	2837467	0.51500	0.48288	-6.237
2	Memantine	5	2	2843904	0.51500	0.48398	-6.023
3	Memantine	5	3	2856054	0.51500	0.48605	-5.621

Peak MemantineX Value (ug/mL): 0.77250

	Name	Level	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	Memantine	6	1	4662628	0.77250	0.79426	2.817
2	Memantine	6	2	4642911	0.77250	0.79089	2.381
3	Memantine	6	3	4640096	0.77250	0.79041	2.319

Peak MemantineX Value (ug/mL): 1.03000

	Name	Level	Injection	Response	X Value (ug/mL)	Calc. Value (ug/mL)	% Deviation
1	Memantine	7	1	6015896	1.03000	1.02513	-0.473
2	Memantine	7	2	6042378	1.03000	1.02965	-0.034
3	Memantine	7	3	6041853	1.03000	1.02956	-0.043

Figure 6. Calibration curve data processed using SIR mass data at 180.2 Da. The percent deviation for the measurements is less than 7.0%.

Sample Analysis

The UPLC-MS method was then used to analyze commercially available memantine HCl tablets, to demonstrate specificity for routine assay. To develop a sample preparation procedure for the tablets, various sample diluents and filters were considered, with a goal of meeting the acceptance criteria for recovery, defined in the USP Monograph for Memantine Hydrochloride Tablets.[4] According to that monograph, "Memantine HCl Tablets contain an amount of memantine hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of memantine hydrochloride ($C_{12}H_{21}N \cdot HCl$)."

Sample diluents investigated during the study are shown in Table 1. Separate sample solutions were prepared by dissolving tablets using all diluents and then sonicating and centrifuging the stock solutions. They were then filtered through 0.2 µm GHP syringe filters before undergoing dilution to the working concentration. The data show that preparation in diluent containing 50:50 0.1 N HCl/ethanol resulted in the highest recovery of memantine HCl: 99.9%.

Tablet prep.	Stock solution diluent	Working solution diluent	% Recovery
1	H_2O	90:10 H_2O/methanol	67.0
2	0.01 M H_3PO_4	0.01 M H_3PO_4	89.4
3	0.05 M H_3PO_4	0.05 M H_3PO_4	91.9
4	0.1 M H_3PO_4	0.1 M H_3PO_4	91.9
5	0.1 N HCl	0.1 N HCl	90.1
6	50:50 0.05 M H_3PO_4/ethanol	0.05 M H_3PO_4	93.7
7	50:50 0.1 N HCl/ethanol	0.1 N HCl	99.9

Table 1. Diluent study for development of a sample preparation procedure for memantine HCl tablets.

In addition to diluent, the effect on recovery of filter type was also evaluated. Particulates or insoluble materials present in the sample solution can interfere with chromatography or effect a poor recovery. Removal of particulates through sample filtration can often improve the recovery. The effect on recovery of three different filter membranes, as compared with no filtration (control), was studied (Table 2). Stock sample solutions prepared in 50:50 0.1 N HCl/ethanol were filtered through three, 0.2-µm, syringe filters. As shown in

Table 2, filtration through the GHP-membrane syringe recovered the memantine HCl from the tablet formulation most effectively. Significantly, recovery of the compound from the unfiltered sample solution was lower than it was from the solution filtered through the GHP filter. These results show that sample filtration and prudent selection of filter type are necessary to maximize recovery.

Syringe filter	Waters P/N	Stock solution diluent	Working solution diluent	% Recovery
unfiltered	N/A	50:50 0.1N HCl/Ethanol	0.1N HCl	94.5
GHP	WAT200562	50:50 0.1N HCl/Ethanol	0.1N HCl	99.8
Nylon	WAT097962	50:50 0.1N HCl/Ethanol	0.1N HCl	93.3
PVDF	WAT200804	50:50 0.1N HCl/Ethanol	0.1N HCl	98.1

Table 2. Syringe-filter study for development of a sample preparation procedure for memantine HCl tablets.

For sample analysis, three separate preparations of tablets were tested for the assay of memantine HCl. An example of the chromatographic data of sample diluent and tablet sample solution acquired using the ACQUITY QDa Detector with SIR at 180.2 Da is displayed in Figure 7. The average percent recovery of memantine HCl for the three preparations ranged from 96.0 to 100.1% (Figure 8), which meets the USP acceptance criteria of 90.0–100.0%, defined in the USP Monograph for memantine HCl tablets.

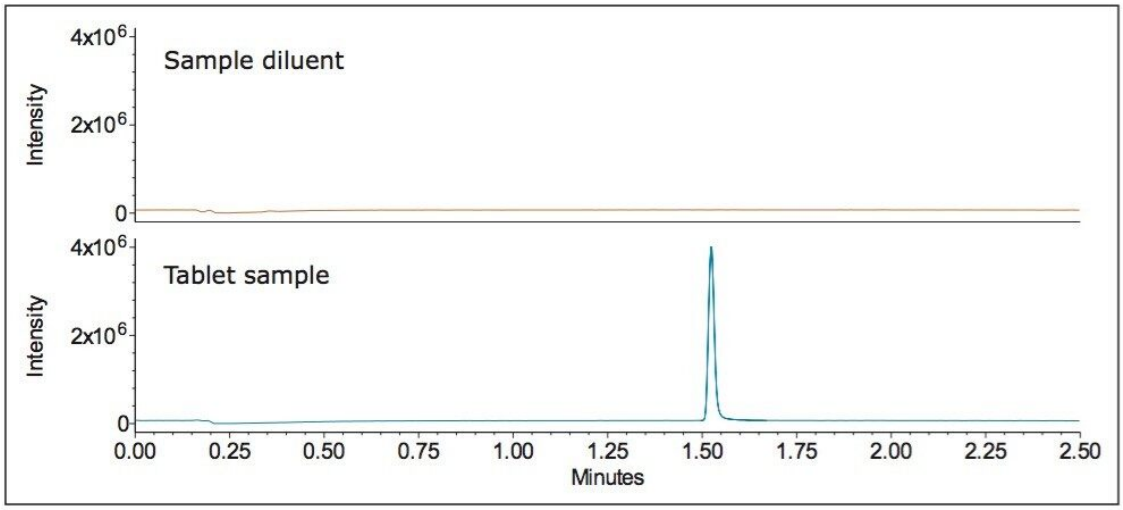
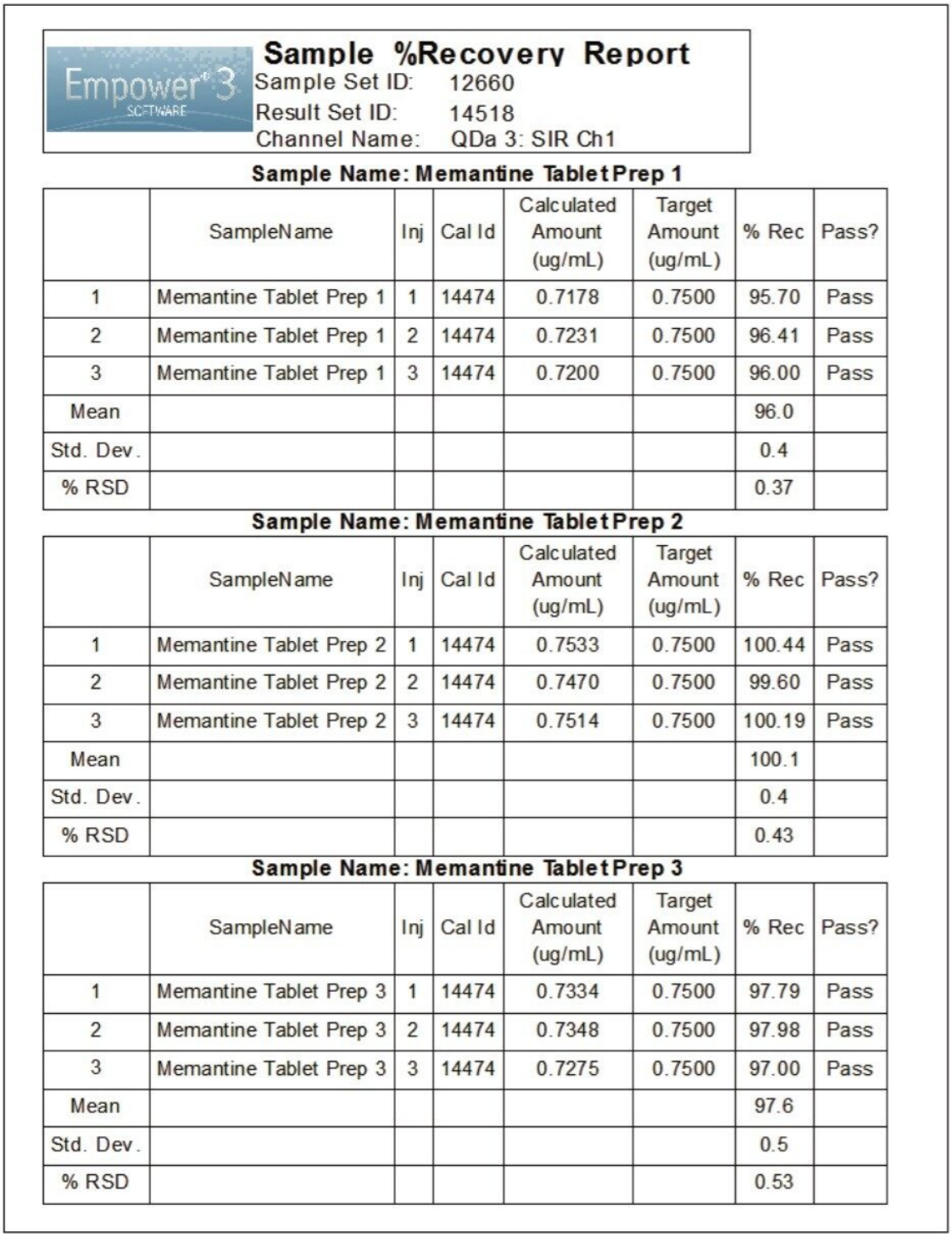


Figure 7. Tablet sample solution analysis for memantine HCl assay, SIR mass data at 180.2 Da.



Sample %Recovery Report

Sample Set ID: 12660
Result Set ID: 14518
Channel Name: QDa 3: SIR Ch1

Sample Name: Memantine Tablet Prep 1

	SampleName	Inj	Cal Id	Calculated Amount (ug/mL)	Target Amount (ug/mL)	% Rec	Pass?
1	Memantine Tablet Prep 1	1	14474	0.7178	0.7500	95.70	Pass
2	Memantine Tablet Prep 1	2	14474	0.7231	0.7500	96.41	Pass
3	Memantine Tablet Prep 1	3	14474	0.7200	0.7500	96.00	Pass
Mean						96.0	
Std. Dev.						0.4	
% RSD						0.37	

Sample Name: Memantine Tablet Prep 2

	SampleName	Inj	Cal Id	Calculated Amount (ug/mL)	Target Amount (ug/mL)	% Rec	Pass?
1	Memantine Tablet Prep 2	1	14474	0.7533	0.7500	100.44	Pass
2	Memantine Tablet Prep 2	2	14474	0.7470	0.7500	99.60	Pass
3	Memantine Tablet Prep 2	3	14474	0.7514	0.7500	100.19	Pass
Mean						100.1	
Std. Dev.						0.4	
% RSD						0.43	

Sample Name: Memantine Tablet Prep 3

	SampleName	Inj	Cal Id	Calculated Amount (ug/mL)	Target Amount (ug/mL)	% Rec	Pass?
1	Memantine Tablet Prep 3	1	14474	0.7334	0.7500	97.79	Pass
2	Memantine Tablet Prep 3	2	14474	0.7348	0.7500	97.98	Pass
3	Memantine Tablet Prep 3	3	14474	0.7275	0.7500	97.00	Pass
Mean						97.6	
Std. Dev.						0.5	
% RSD						0.53	

Figure 8. Percent recovery of memantine HCl from the tablet samples, SIR mass data at 180.2 Da. The USP acceptance criteria for %Recovery specified in the USP Monograph for Memantine HCl tablets are: 90.0–100.0%.

Conclusion

Mass detection using the ACQUITY QDa Detector enabled detection and quantitative determination of non-chromophoric memantine HCl. System suitability and method linearity calculated using mass data were excellent. For analysis of tablets, a non-derivatization sample preparation procedure for quantitative determination of memantine HCl was developed, eliminating the need for a complex and tedious pre-column derivatization protocol before the analysis. The use of SIR allowed the selection of a target mass, reducing interferences in the analysis of formulated samples.

Overall, the ACQUITY QDa Mass Detector is a robust, simple to use, orthogonal, detection technique to UV detection. It provides accurate and reliable results, making this technology ideal for routine testing in the QC laboratory.

References

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