AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Development of LC-MS Method for Characterization of Drotaverine Hydrochloride Impurities

Dileep M. Bhosale[1] and Anna Pratima G. Nikalje*[1]
1.Department of Pharmaceutical Chemistry, Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad, M.S., India, 431001

ABSTRACT

A novel, simple and rapid reversed-phase liquid chromatography mass spectrometric method (LC-MS) was developed and subsequently used for the characterization of Drotaverine hydrochloride (DRH) and its impurities. The separation was achieved in 22 minutes on Merck Purosphere STAR RP-18e (250 x 4.6) mm, 5 µm column in gradient mode with flow rate 1.5 mL/min. 0.05 M ammonium acetate buffer pH 3.0 and a mixture of acetonitrile and methanol 85:15 v/v was used as mobile phase A and mobile phase B, respectively. Detection was carried out at the optimum wavelength of 280 nm using a photodiode array/triple quadrupole mass detectors. The retention time of Drotaverine was found about 7 minutes. Specificity of the method was established by blank solution and the extreme degraded sample was used for the detection of impurity masses. The impurities detected under mass detector were further ionized for their daughter ions.

Keywords: Drotaverine Hydrochloride (DRH), Impurities, LC-MS, Daughter Ions, Characterization,

*Corresponding Author Email: annapratimanikalje@gmail.com
Received 28 May 2016, Accepted 05 June 2016

Please cite this article as: Pratima AJ *et al*., Development of LC-MS Method for Characterization of Drotaverine Hydrochloride Impurities. American Journal of PharmTech Research 2016.

INTRODUCTION

Drotaverine Hydrochloride (DRH) with molecular mass 433.97 g/mol[1] is chemically 1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline and is a highly potent spasmolytic agent[2]. It acts as an antispasmodic agent specific for smooth muscle spasms and reduces excessive labor pains[2]. Literature survey revealed that few analytical methods have been published for the determination of DRH and its related impurities by reversed phase chromatography.[2-10] Those published methods were suitable either for quantification of DRH alone or in combination with other drugs. However, the exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals published the LC-MS method for characterization of DRH and its impurities by reversed phase chromatography. So we successfully developed liquid chromatography mass spectrometric procedure with 22 minutes run time which will serve as a common tool for the identification of DRH impurities from bulk and pharmaceutical products. The chemical structure of DRH is presented in figure 1.

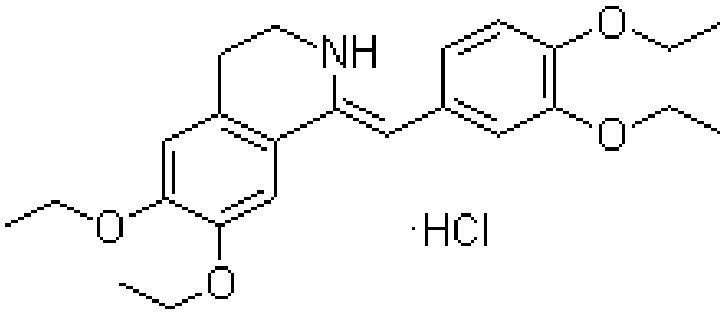

Figure 1: Chemical structure of DRH

MATERIALS AND METHOD

Chemicals and Reagents

Ammonium acetate (GR grade), Glacial acetic acid (GR grade), Acetonitrile (HPLC grade) and Methanol (HPLC grade) were purchased from Merck Fine Chemicals Ltd. (Mumbai, India). The 0.45µm nylon-66 filters were purchased from M/s Advanced Micro Devices Pvt Ltd., India. High purity Drotaverine hydrochloride (DRH) was gifted by M/s Simson Pharma, Mumbai. Ultrapure water (15 MΩ cm at 25°C) was prepared by using Elix model of Merck Millipore water purification system, Germany and was used throughout the experiment. Other chemicals used were of AR or GR grade.

Chromatograpic conditions

The Waters Alliance high performance liquid chromatographic system was used comprised of degasser, quaternary pump, auto injector, column compartment with heater & chiller facility,

photodiode array/mass detector and system control. Data collection and data processing were accomplished by using Waters Empower-2 chromatography data software. The separation of DRH and its degradent products was achieved on Merck Purosphere STAR RP-18e (250 x 4.6) mm, 5µm column in gradient mode. Mobile phase A consisted of 0.05M ammonium acetate buffer pH 3.0 and mobile phase B consisted of a mixture of acetonitrile and methanol in ratio 85:15 v/v. The mobile phase pumped through column with flow rate of 1.5 mL/min in gradient mode. Injection volume was kept 20 µL throughout the study. Based on the response of DRH and degradent peaks response, the optimum wavelength 280 nm was selected. The pump gradient programme is presented in Table 1.

Table 1: Pump Gradient Programme

Time (Minutes)	% Mobile phase A	% Mobile phase B
0	60	40
12	45	55
18	60	40
22	60	40

Blank Preparation

Mobile phase was used as a blank solution throughout the study. Blank chromatogram is presented in figure 2.

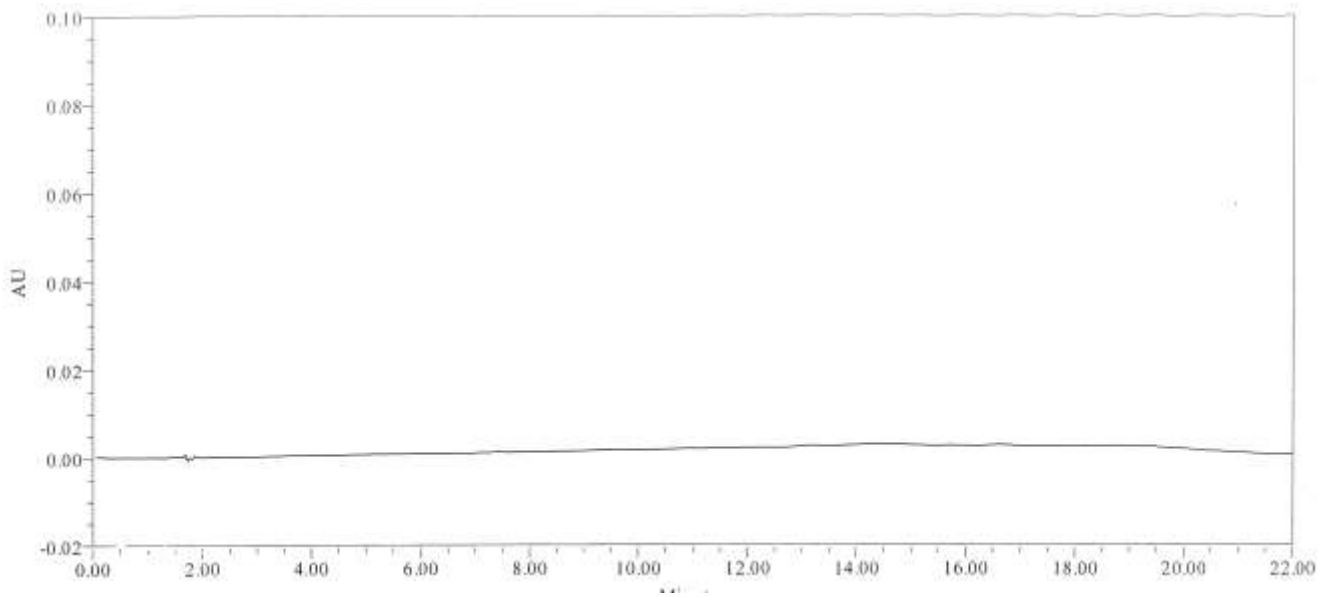

Figure 2: Typical chromatogram of Blank

Sample solution preparation

Dissolved sample equivalent to about 50 mg of DRH into 50 mL mobile phase by ultra sonication and mixed to achieve the concentration of DRH about 1000 µg/mL. This solution was filtered

through 0.45µ nylon-66 membrane syringe filter by discarding first 2-3 mL of filtrate. The liquid chromatogram of degraded sample is presented in figure 3.

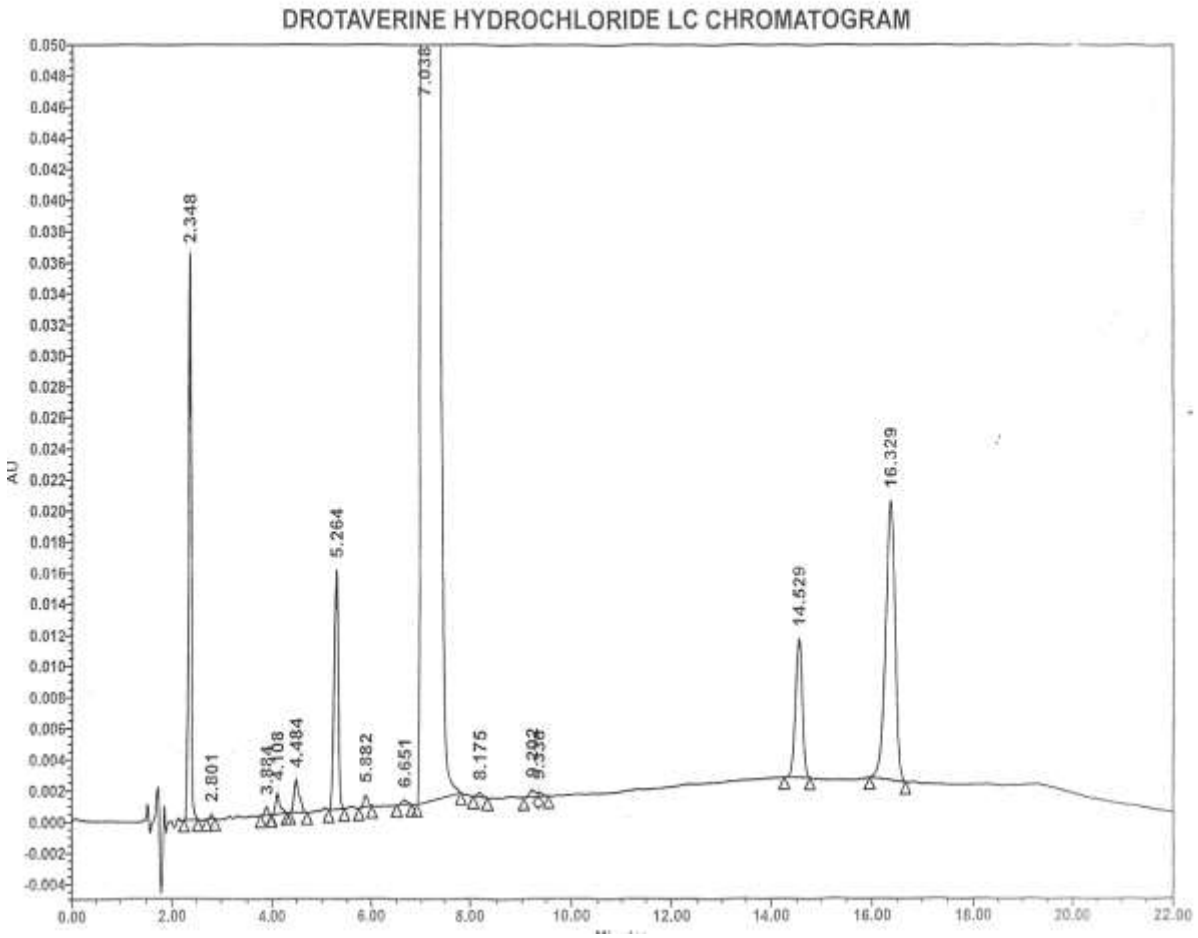

Figure 3: Typical LC chromatogram of degraded sample

Forced degradation studies

For hydrolysis degradation the sample solutions containing DRH 1000µg/mL were treated with 5 mL 1N hydrochloric acid and 5 mL 1N sodium hydroxide for 1 hr on water bath at 70°C. All the solutions prepared were quenched to their original pH. For oxidation degradation the sample solution was treated with 5 mL of 5% v/v Hydrogen Peroxide and exposed to 70°C on water bath for 1 hr. The above hydrolysis and oxidation samples pooled together in ratio 1:1:1 and analyzed by high performance liquid chromatography coupled with photo diode array detector to check the peak purity indices. All peaks showed purity angle less than purity threshold, thus proved the homogeneity of the peaks of interest. The peak purity data is presented in Table 2. Also, the same sample analysis was carried out by LC-MS using electron ionization source and triple quadrupole detector. The data obtained scanned for the mass range 100-800 Da by using mass spectrometer. The degradents mass observed are further ionized to study the daughter ions.

Table 2: Peak purity profile of DRH degradents

Retention Time (min)	Purity Angle	Purity Threshold	Purity Flag
2.248	1.153	1.251	No
2.801	3.452	6.485	No
3.884	3.224	4.675	No
4.108	1.278	2.436	No
4.484	5.588	6.059	No
5.264	0.684	1.485	No
5.882	2.364	3.187	No
6.651	11.942	12.683	No
7.038	0.475	1.004	No
8.175	17.889	19.873	No
9.202	2.938	3.230	No
9.339	5.001	5.395	No
14.529	1.285	1.829	No
16.329	0.181	1.196	No

The LC and total ionization chromatogram of DRH and degradents are presented in figure 4. The mass scan for the available signals is presented in figure 5-6. The mass scan for individual degradents is presented in figure 7-8 and the respective daughter ions are presented in figure 9-10.

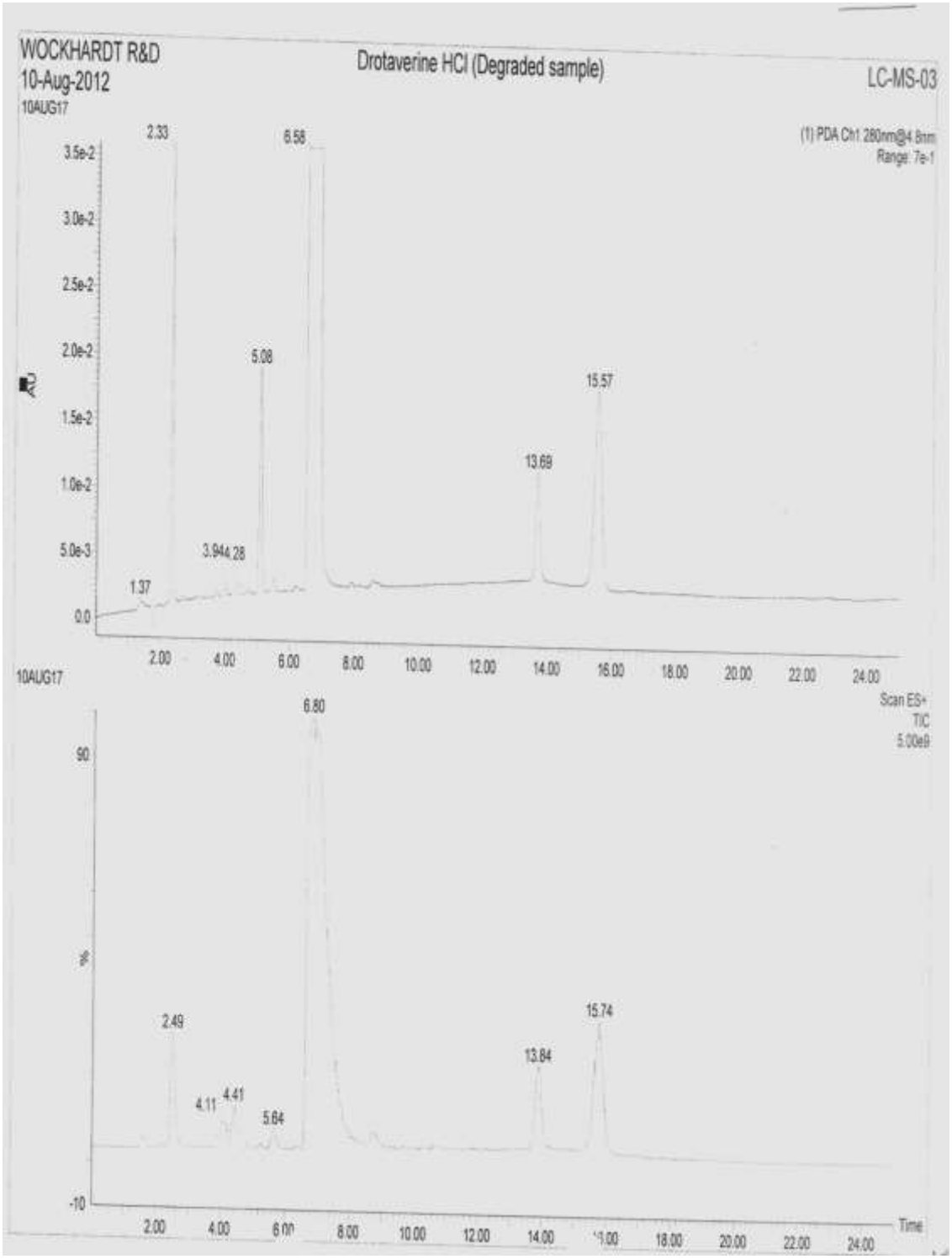

Figure 4: LC and total Ionization chromatogram of DRH sample

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The mechanism of retention in the reverse phase packing is due to the partitioning of the molecule into the lipophylic stationary phase, which primarily depends upon the lipophlicity of the

compound. The other factor that influences the degree of retention is the nature of the mobile phase. The reversed-phase chromatography allows efficient separation of substances with different polarities by altering the composition/polarity of the mobile phase. Retention, selectivity and peak symmetry of compounds are strongly influenced by the sorbents/stationary phases. Strongly distorted peaks of the compounds are often observed when unsuitable RP sorbents are used, due to the interaction of the compounds with free silanol groups on the sorbent matrix. DRH is a strong basic compound. The chemical structure of DRH is presented in figure 1. The main objective of the investigation was to develop a reliable method on reverse phase stationary phase which will be helpful to identify and characterize the impurities present in the sample. We initiated the development by using the Merck Purosphere STAR RP-18e column on HPLC by using ammonium acetate as a buffer in mobile phase and methanol/acetonitrile as organic modifier in a gradient mode of 70 minutes. Based on the retention behavior of the peaks, we optimized the gradient to achieve elution of all impurity peaks within 18 minutes followed by 4 minutes reconditioning of the column. The optimum performance of chromatography was achieved in 0.05M ammonium acetate buffer pH 3.0 as mobile phase A and acetonitrile and methanol 85:15 v/v as mobile phase B with 1.5 mL/min flow in gradient mode. The 280 nm wavelength of analysis was selected based on the optimum response of the impurity peaks.

The pump gradient programme is presented in Table 1 and the peak purity data is presented in Table 2. The purity angle found less than the purity threshold for all peaks, thus proving the homogeneity of the analyte peaks. The blank chromatogram of liquid chromatogram is presented in figure 2 and the degraded sample is presented in figure 3. The baseline separation was observed for all major degradents in liquid chromatogram.

The pooled forced degradation sample prepared under liquid chromatography was analyzed by LC-MS using electron ionization source and triple quadrupole detector. The data obtained scanned for the mass range 100-800 Da by using mass spectrometer. The degradents mass observed are further ionized to study the daughter ions.

The LC and total ionization chromatogram of DRH and degradents are presented in figure 4. The mass scan for the available signals is presented in figure 5-6. The mass scan for individual degradents is presented in figure 7-8 and the respective daughter ions are presented in figure 9-10. The mass fragmentation and daughter ions observed under this study confirms the probable molecular weights of the impurities generated in the sample.

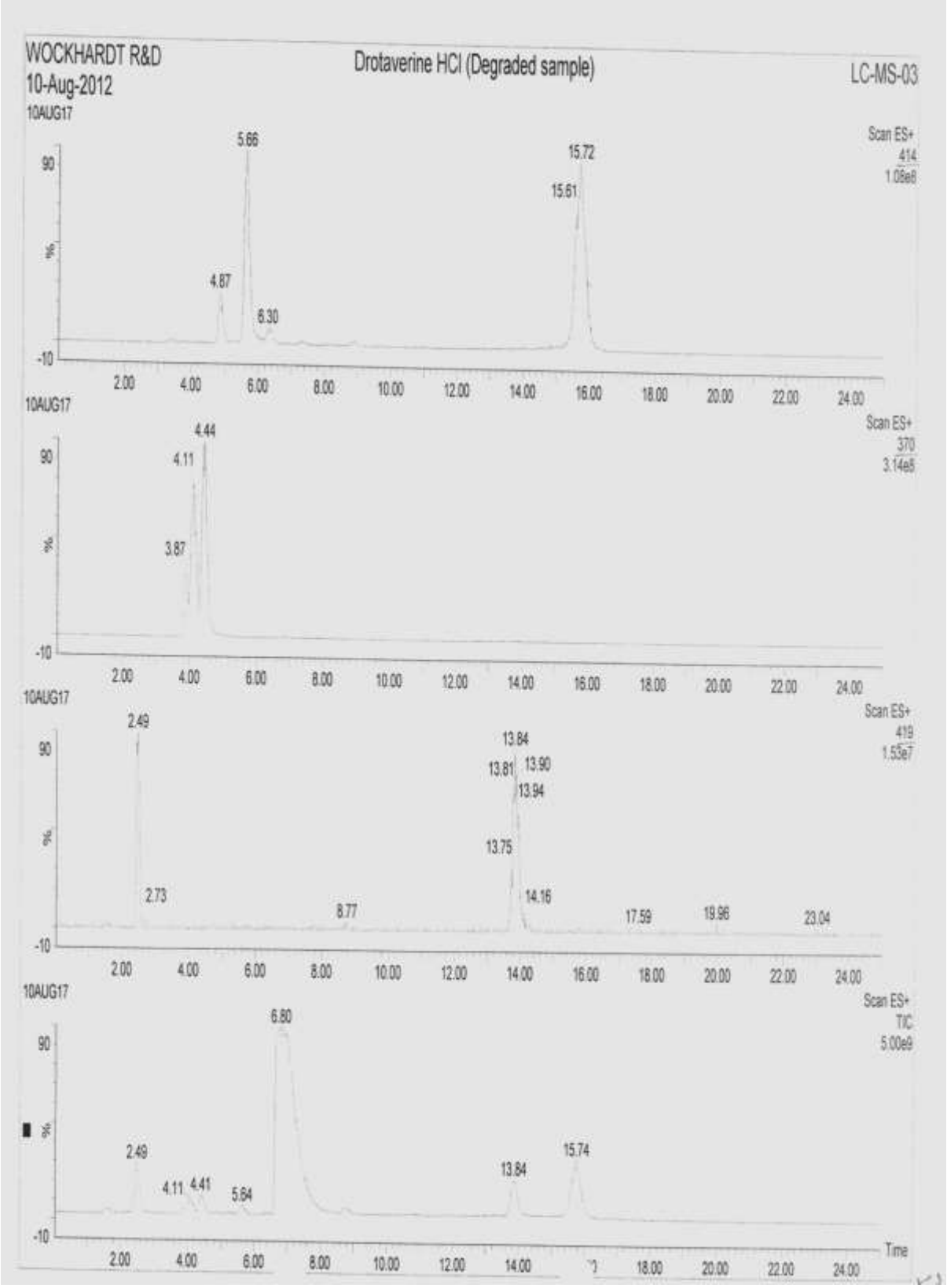

Figure 5: Mass scan of DRH sample Part-I

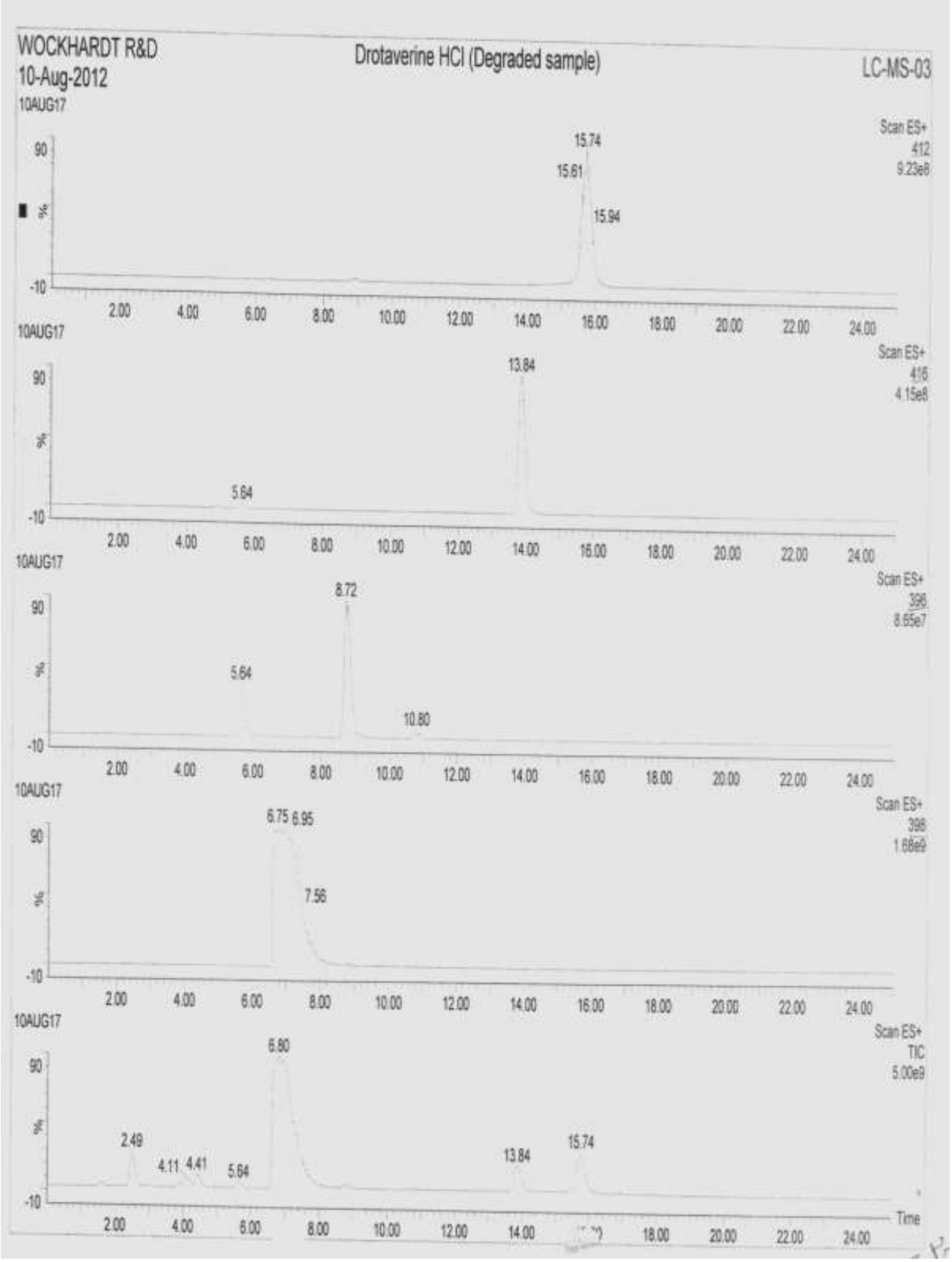

Figure 6: Mass scan of DRH sample Part-II

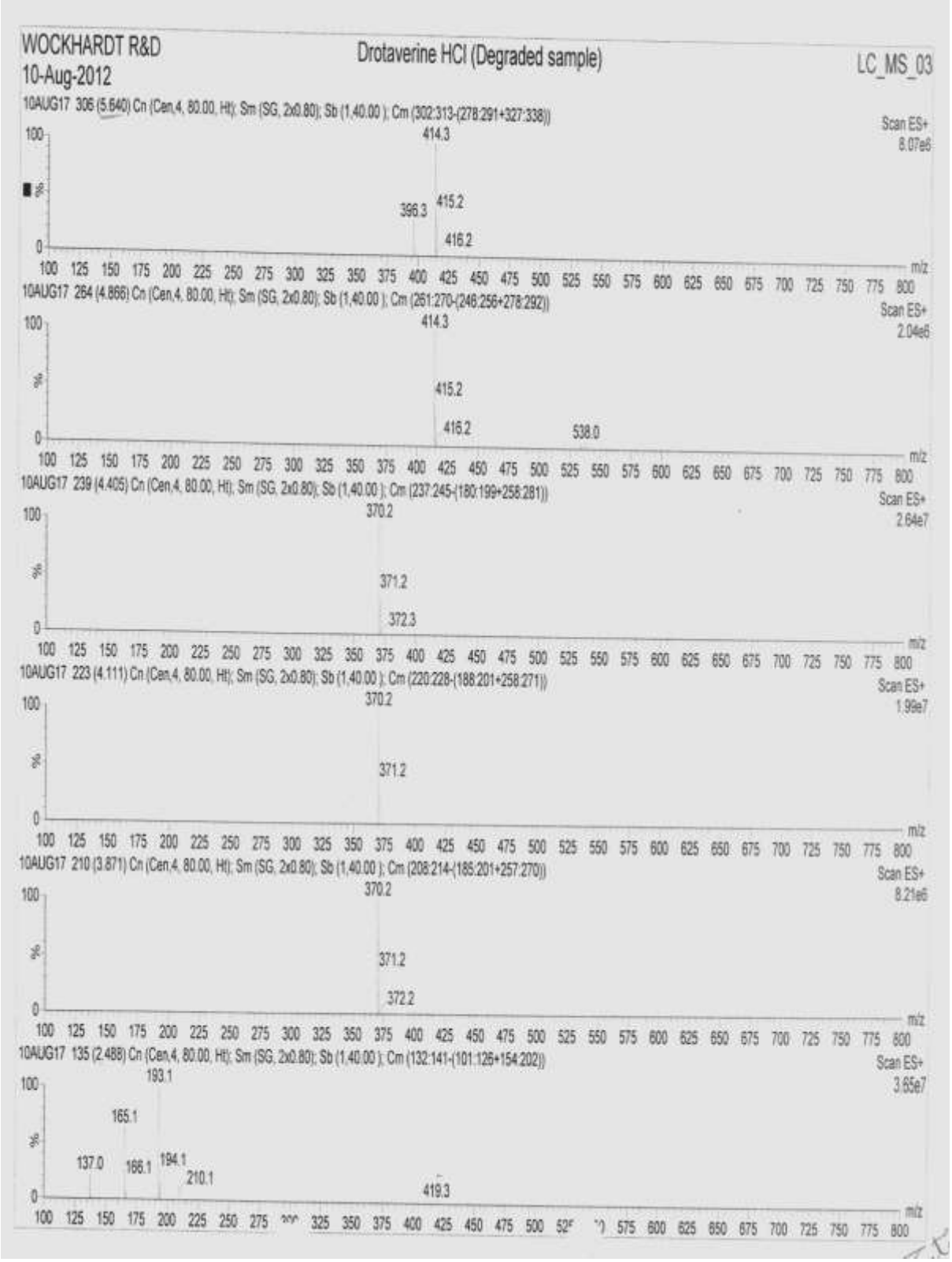

Figure 7: Mass of individual degradent of DRH sample Part-I

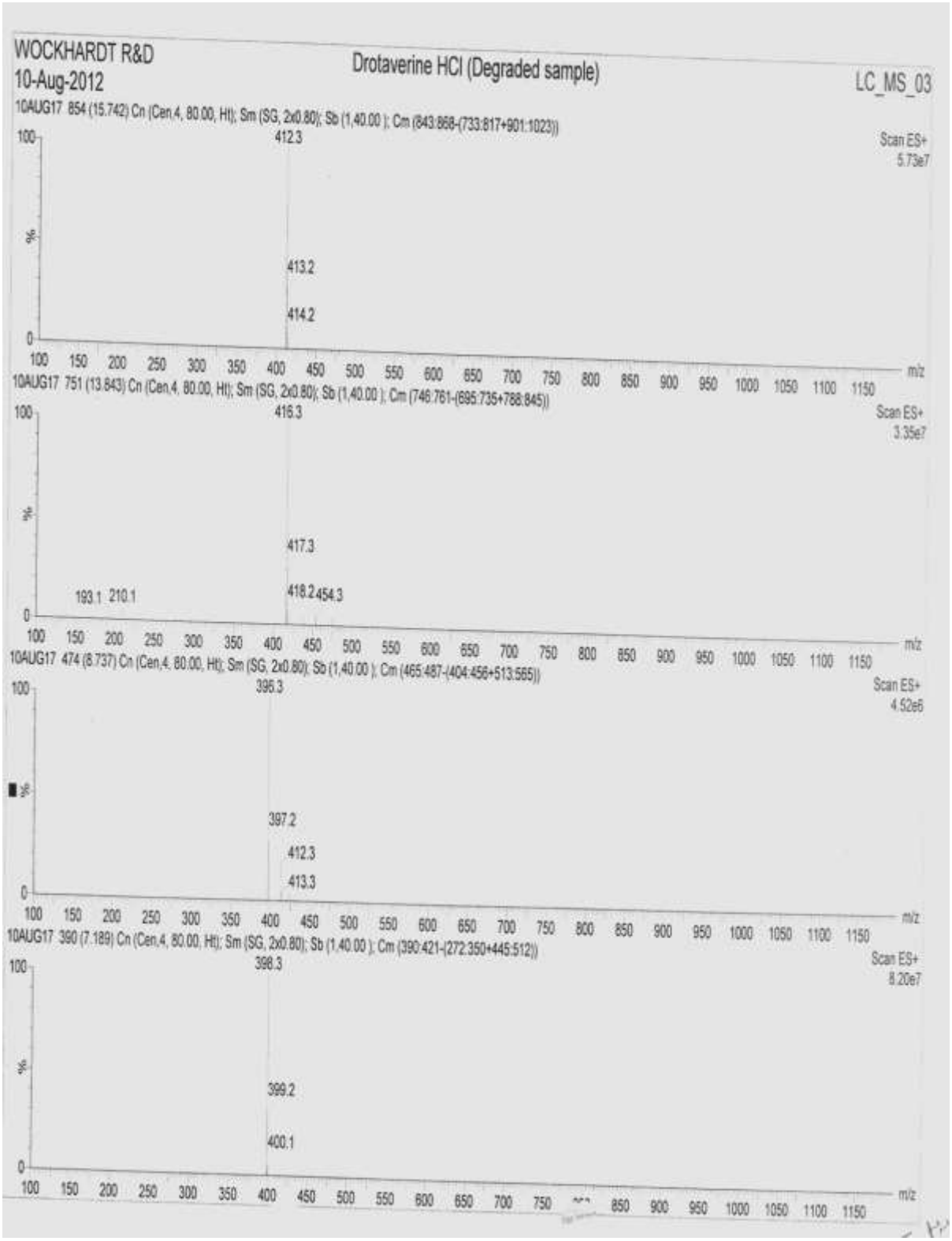

Figure 8: Mass of individual degradent of DRH sample Part-II

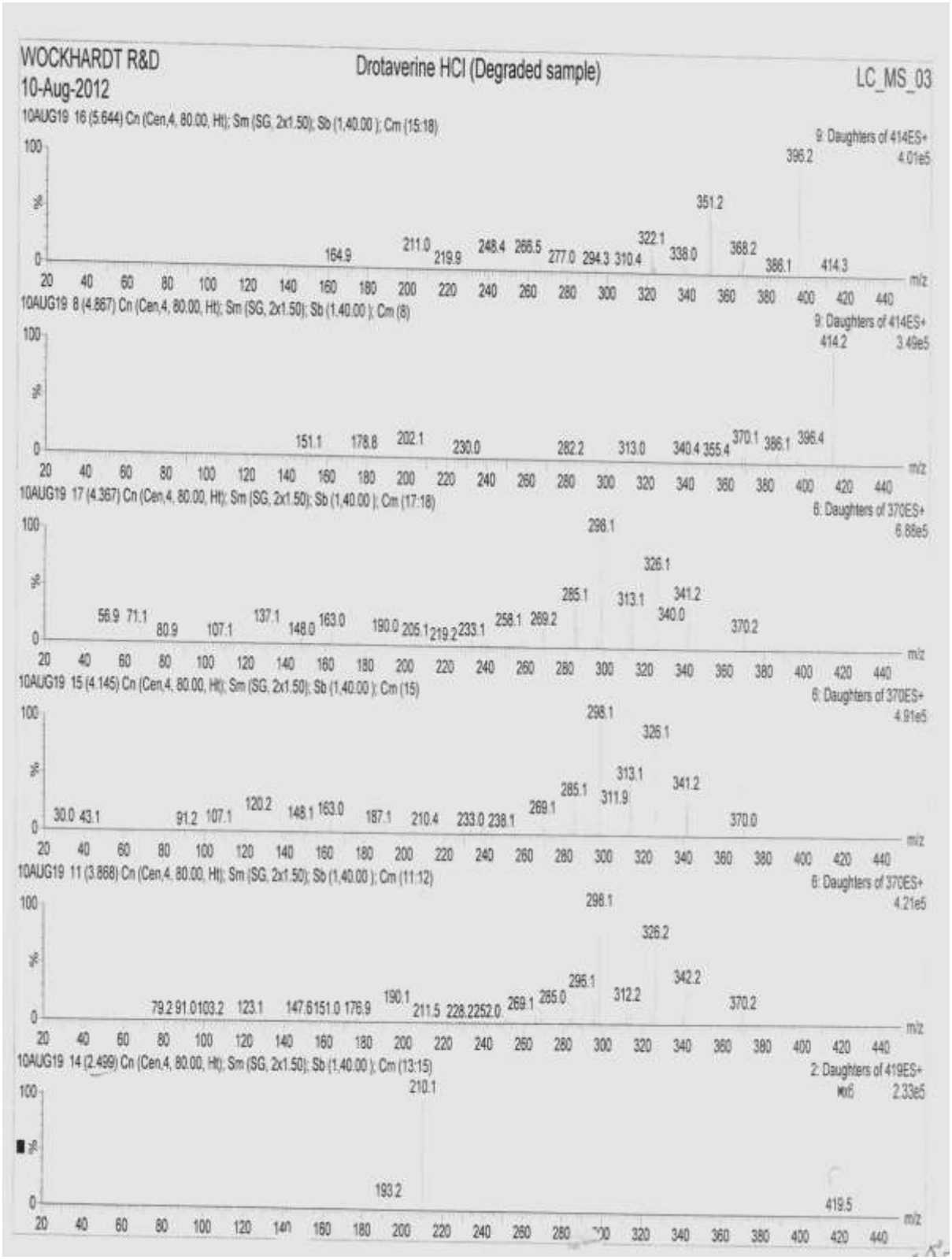

Figure 9: Daughter ions of individual mass Part-I

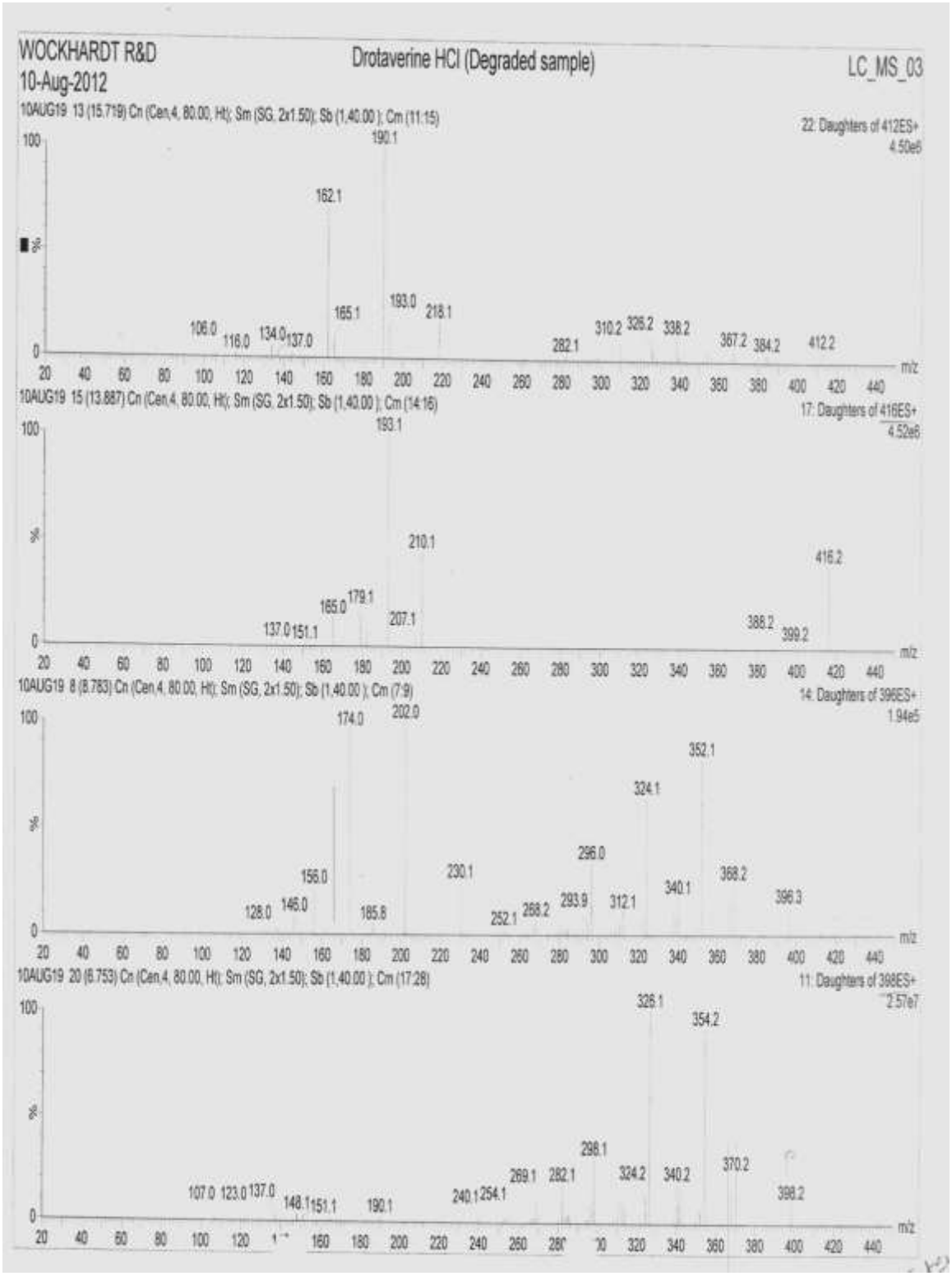

Figure 10: Daughter ions of individual mass Part-II

CONCLUSION

A new RP-LC/MS method with 22 minutes run time was successfully developed and employed for the identification and characterization of Drotaverine hydrochloride and its impurities from drug

substance/drug product matrix. The proposed method provides optimum selectivity between the analyte peak and the degradants formed under degradation study. Moreover, this method can also be used for the other dosage forms after establishment of the specificity studies. This method will serve as a tool for the researchers for the identification and characterization of Drotaverine hydrochloride unknown impurities present in the samples.

ACKNOWLEDGEMENTS

Authors are grateful to the Chairman, Mrs. Fatma Rafiq Zakaria, Maulana Azad Education Trust and Dr. Zahid Zaheer, Principal, Y.B Chavan College of Pharmacy, Aurangabad for encouragement and support. The authors would like to thank M/s Simson Pharma, Mumbai for providing the gift samples to conduct this research.

REFERENCES

1. http:/www.chemblink.com
2. Thummala VR, Tharlapu SS, Rekulapalli VK, Ivaturi MR, Nittala SR. Development and Validation of a Stability-Indicating RP-HPLC Method for the Estimation of Drotaverine Impurities in API and Pharmaceutical Formulation Sci Pharm 2014;82:99-115.
3. Panigrahi D, Mishra GP, Sharma R. Study of stressed degradation behavior of drotaverine and development of a validated stability-indicating HPLC assay method Der Pharma Chemica 2012;4:847-853.
4. Rekulapally VK, RAo VU, Kumar NA, Subbaiah BV A Novel, Rapid, and Validated Stability-Indicating UPLC Method for the Estimation of Drotaverine Hydrochloride and Ibuprofen Impurities in Oral Solid Dosage Form Sci Pharm 2015;83:567–581.
5. Sureshkumar S, Kalyankumar, Natraj KS, Rao VJ Development and validation of RP-HPLC method for simultaneous estimation of Drotaverine hydrochloride and Paracetamol in tablet dosage form J chem. Pharm sci 2011;4:58-61
6. Jain JR, Shah DR, Shah SA, Chauhan RS RP-HPLC method for simultaneous estimation of Drotaverine hydrochloride and Aceclofenac in their combined tablet dosage form Der Pharma Chemica 2011;3:245-252.
7. Muralidharan S Simple and Sensitive Method for the Simultaneous Estimation of Drotaverine HCL and Aceclofenac Using RP-HPLC J Pharma Sci Drug Des 2014;1:1-3.
8. Shah SA, Shah DR, Chauhan RS, Jain JR Development and Validation of Simultaneous Spectrophotometric Methods for Drotaverine Hydrochloride and Aceclofenac from Tablet Dosage Form Indian J Pharm Sci 2011; 73: 296–300.

9. Durgam RB Analytical method development and validation of Drotaverine Hydrochloride and Aceclofenac in bulk and pharmaceutical dosage forms by UV-Spectrophotometer Int J Drug Dev & Res 2013;5:268-272.
10. Mahaparle S, Telekone RS, Raut RP, Damle, Kasture PV Simultaneous Spectrophotometric Determination of Drotaverine Hydrochloride and Paracetamol in Tablet Indian Journal of Indian J. Pharm. Sci 2010;72: 133-136.