

1      Development and Validation of RP-HPLC Method for  
2      Simultaneous Determination of Guaifenesin Impurities in Multi  
3      Drug Combinations

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9      **Abstract**

10     A High Performance Liquid Chromatographic method was developed and validated for  
11     quantitative determination of Guaifenesin impurities including 2-(2-  
12     methoxyphenoxy)propane-1,3-diol (?-isomer) and 2- m ethoxyphenol (guaiacol) in different  
13     multi drug components pharmaceutical dosage forms, containing guaifenesin, ambroxol  
14     hydrochloride and salbutamol sulfate . The different analytical performance parameters such  
15     as linearity, precision, accuracy, limit of detection (LOD), limit of Quantification (LOQ) were  
16     determined according to International Conference on Harmonization (ICH) Q2B guidelines.  
17     The chromatographic separation was achieved on EC NUCLEODUR-100-3C18 (250x4,6 mm,  
18     5?m packing) column using gradient elution of Solvent A (0.1 M ammonium acetate buffer of  
19     pH 6.8) and solvent B (acetonitrile : methanol (80:20)) The Ultra Violet spectrophotometric  
20     determination was performed at 275 nm. The Linearity of the calibration curves for the  
21     analytes in the desired concentration range is good ( $r^2 = 0.999$ ) by High Performance Liquid  
22     Chromatography. The LOQ were 1 and 0.1 ?g/ml respectively for guaifenesin ?-isomer and  
23     guaiacol. The average percentage recovery of guaifenesin impurities was found to be within  
24     98.6 ???" 101.2

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26     **Index terms**— RP-HPLC, validation, guaifenesin impurities, 2-(2-methoxyphenoxy)-propane-1, 3-diol (?-  
27     isomer) and 2- methoxyphenol (guaiacol).

28     **1 Introduction**

29     ncreased mucus secretion is a clinical feature of severe respiratory diseases, such as asthma, cystic fibrosis  
30     and chronic obstructive pulmonary disease [1]. Pharmacological approaches for relieving mucus hypersecretion  
31     currently include several classes of agents, including expectorants, mucoregulators, mucolytics, bronchodilators  
32     anti-inflammatory drugs and antioxidants [2]. Classic mucolytic drugs such as Nacetylcysteine decrease the  
33     viscoelastic properties of mucus by reducing disulfide bonds. In contrast, expectorants change mucus consistency  
34     and make coughing more productive, mucokinetics improve transportability, and mucoregulators suppress mucus  
35     secretion. Mucolytics generally decrease mucus viscosity by reducing the dicysteine bridges that contribute to  
36     the rigidity of the mucins [3]. Guaifenesin (GFN) is a commonly used expectorant drug for productive cough,  
37     which is reported to increase the volume and reduce the viscosity of tenacious sputum [4,5].

38     Currently recommend consideration for management of mucus hypersecretion is the combination of expecto-  
39     rants, mucoregulators, mucolytics and even bronchodilators in different multi drug components pharmaceutical  
40     formulations [3,6]. Therefore, the simultaneous identification and quantification of active pharmaceutical ingredi-  
41     ents (API) and its related impurities along with some other active ingredients and excipients in multicomponent

## 8 G) SPECIFICITY AND ROBUSTNESS

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42 pharmaceutical products is a very intensive activity performed at many levels of the drug discovery pipeline and  
43 beyond. Impurities relate to starting materials, byproducts, breakdown products or polymorphs are of significant  
44 concern as they may carry activity responsible for eventual undesirable side effects or toxicity and may interfere  
45 with the drug's activity. Thus monitoring impurities in API which exist as various combinations in cough-cold  
46 multicomponent drug products is a prerequisite for insuring drug safety and quality.

47 A literature survey reveals some HPLC methods that are reported for the simultaneous determination of GFN  
48 along with some other active ingredients in a multicomponent tablet and liquid dosage formulation as anticipated  
49 with the variation of mobile phase, column and detector. Different HPLC methods for individual assay and  
50 related impurities are available for GFN in official pharmacopoeia and several LC-MS/MS methods were used  
51 for determination of GFN in Human Plasma [11]. Hence an attempt has been made to develop a simple, efficient  
52 and selective method for the determination of guaifenesin impurities (Figure 1), 2-(2methoxyphenoxy)-propane-  
53 1,3-diol ( -isomer) and 2-methoxyphenol (guaiacol) in the presence of guaifenesin, ambroxol hydrochloride and  
54 salbutamol sulfate in multi drug components pharmaceutical formulations.

## 55 2 II.

## 56 3 Materials and Methods

### 57 4 a) Instrumentation

58 A High Performance Liquid Chromatography (HPLC) method for GFN -isomer and guaiacol analytical method  
59 was developed on PLATIN BLUE UPLC (Knauer, Germany) with diode array detector. NUCLEODUR-100-  
60 3C18 (250x4, 6 mm, 3 m packing, Machery-Nagel, Germany) column was used. The elution was carried by  
61 gradient elution method of mobile phases A and B.

### 62 5 b) Chemicals

63 Ammonium acetate (Sigma-Aldrich, HPLC grade), Millipore water, methanol (HPLC grade, Alpha chemika,  
64 purity: 99.9%, batch: A---); acetonitrile (HPLC grade, Alpha chemika; purity: 99.9%, batch: A5982;), impurity  
65 A, 2-methoxyphenol (guaiacol) Sigma-Aldrich, purity: 99,9%); impurity B 2-(2-methoxyphenoxy)propane-1,3-diol  
66 ( -isomer) (Sigma-Aldrich, purity: 99,9%); GFN and ambroxol hydrochloride (Sigma-Aldrich, purity: 99,9%);  
67 salbutamol sulfate (Sigma-Aldrich, purity: 99,8%); methylparaben (Sigma-Aldrich, purity: 100%); propylparaben  
68 (Sigma-Aldrich, purity: 100%) and citric acid monohydrate (Sigma-Aldrich, purity: 99.7%) were used in this  
69 study.

### 70 6 c) Preparation of stock solution and working standard solu- 71 tion

72 Preparation of mobile phase. Solvent A -7.7 gm of ammonium acetate was weighed and transferred into a 1000  
73 ml beaker, dissolved and diluted with 1000 ml water and pH brought to 6.8 by ammonia or acetic acid. The  
74 solvent A was filtered through 0.45 m membrane filter under vacuum filtration and was degassed before used, then  
75 delivered at a flow rate 1.0 ml/min. Solvent B -acetonitrile and methanol (80:20). d) Preparation of solvent for  
76 standards and sample Solvent C-750 ml of solvent A and 250 ml of solvent B are mixed together. e) Preparation  
77 of standard solution 10.0 mg of GFN standard was weighed and transferred into 10 ml volumetric flask. 8 ml of  
78 solvent C was added sonicated for 5 min, mixed thoroughly to dissolve and make up the volume to 10 ml with  
79 mobile phase (1 mg/ml concentration). 5.0 mg of guaiacol reference standard was weighed and transferred into 20  
80 ml volumetric flask and make up the volume to 20 ml with solvent C. 1.0 ml of guaiacol solution was transferred  
81 into 50 ml volumetric flask and make up the final volume to 50 ml with solvent C (5 g /ml concentration). 10.0  
82 mg of GFN -isomer reference standard was weighed and transferred into 20 ml volumetric flask and make up the  
83 volume to 20 ml with solvent C. 1.0 ml of GFN -isomer solution was transferred into 50 ml volumetric flask and  
84 make up the final volume to 50 ml with solvent C (10 g/ml concentration).

### 85 7 f) Preparation of sample solution

86 Melon® (Aversi, Georgia) which is a combination of ambroxol hydrochloride (15 mg); salbutamol sulfate (2.4 mg);  
87 guaiphenesin (100 mg), per 190 mg tablet or a cough mixture of ambroxol hydrochloride (15 mg); salbutamol  
88 sulfate (1.2 mg); guaiphenesin (50 mg), per 5 mL syrup were used in this study. 20 tablets were grinded in to  
89 a homogenous powder and 190 mg were transferred into 100 ml volumetric flask and make up the final volume  
90 to 100 ml with solvent C (1mg/ml concentration). 10.0 ml of the syrup was transferred into 100 ml volumetric  
91 flask and make up the final volume to 100ml with solvent C (1mg/ml concentration). The sample solutions were  
92 sonicated for 5 min, mixed thoroughly to dissolve and filtered through 0.45 m membrane filter.

### 93 8 g) Specificity and Robustness

94 The specificity of the assay method is established by injecting blank, containing 1 mg/ml GFN, ambroxol  
95 hydrochloride, salbutamol sulfate methyl-, propylparaben and citric acid monohydrate as well as standard

96 and samples into the HPLC. The identity of GFN impurities, including -isomer and guaiacol was confirmed  
97 by comparison of its retention time (RT) and UV-spectra. Robustness was established by varying the  
98 chromatographic condition with respect to specificity of the method in various pH conditions of mobile phase.  
99 Standard and sample solutions were injected and the chromatograms were recorded. h) Quantification Limits

100 The quantification limit was defined as the lowest fortification level evaluated at which acceptable average  
101 recoveries were achieved and analyte peak is Where 'S' is the standard deviation of replicate determination  
102 values; 'K' is the sensitivity namely the slope of the calibration graph.

## 103 **9 i) Calibration curve**

104 The calibration curve was constructed by plotting peak area concentration of GFN impurities standard solutions.  
105 Aliquots of guaiacol standard stock solutions in the concentration range 0.1-10 g/ml and GFN -isomer reference  
106 standard in the concentration range 1.0 -100 g/ml were transferred into 25 ml volumetric flask and 10 ml of  
107 solvent C was added, sonicated for 5 min, mixed thoroughly to dissolve and make up the volume to 25 ml with  
108 solvent C. Each concentration of the standard solutions 10 l was injected and the chromatograms were recorded.  
109 The calibration graph was done by external standard calibration and confirmed using back calculation method.

## 110 **10 j) Accuracy**

111 Accuracy was determined for standard quality samples (in addition to calibration standard) prepared in triplicates  
112 at different concentration levels (5.0, 50, 100  $\mu$ g/ml for GFN -isomer and 0.5, 5.0, 10.0  $\mu$ g/ml for guaiacol standard  
113 solutions respectively.) within the range of linearity of GFN impurities. The results of analysis of recovery studies  
114 were obtained by method validation by statistical evaluation.

## 115 **11 k) Precision**

116 The precision of the instruments was checked by repeatedly (intra day) intermediate (inter day) and reported as  
117 % RSD for a statistically significant number of replicate measurements. Repeatability and intermediate precision  
118 of the method were determined by analyzing 6 samples of the test concentration 5.0, 50, 100  $\mu$ g/ml for GFN  
119 -isomer and 0.5, 5.0, 10.0  $\mu$ g/ml for guaiacol standard solutions respectively.

## 120 **12 l) Stress Conditions**

121 The stress conditions employed for degradation study included oxidative hydrolysis and photochemical degrada-  
122 tion as it described in [12]. To 10 ml of both GFN standard solution and pharmaceutical formulations 10 ml of 1  
123 % v/v H<sub>2</sub>O<sub>2</sub> was added separately. These mixtures were refluxed separately for 1 hour at room temperature. The  
124 forced degradation in oxidative media was performed in the dark in order to exclude possible photo-degradation.  
125 For carrying out photolysis studies the samples were treated with UV light for 6 hours at 254 nm and also in  
126 sunlight.

## 127 **13 III.**

## 128 **14 Results and Discussion**

## 129 **15 a) Method development**

130 The aim of this study was to develop a simple, efficient and selective method for the determination of GFN  
131 impurities 2-(2-methoxyphenoxy)-propane-1,3-diol ( -isomer) and 2-methoxyphenol (guaiacol) in the presence of  
132 GFN, ambroxol hydrochloride and salbutamol sulfate in multi drug components pharmaceutical tablet and syrup  
133 formulations. Various attempts were made to separate all degradation products with different pH of the mobile  
134 phase buffer and composition of methanol in the mobile phase using C-18 and C-8 stationary phase columns.  
135 The RP-HPLC method for GFN -isomer and guaiacol was optimized (Table 1). To ensure great resolution  
136 between all known and unknown degradation compounds, the C-18 stationary phase with an end-capping was  
137 used. HPLC parameters, such as detection wavelength, ideal mobile phase & their proportions and flow rate  
138 were carefully studied (Table 1). After trying different ratios of mixtures of methanol:acetonitrile and ammonium  
139 acetate buffer the best results were achieved by using a gradient elution. The mobile phase gradient constituted  
140 by ammonium acetate buffer: (solvent A) and acetonitrile: methanol (80:20) (solvent B). At a flow rate of 1.0  
141 ml/min, the retention time were 6, 32 min for guaiacol and 12, 73 min for GFN -isomer. The analytes peak  
142 areas were well defined and free from tailing under the described experimental conditions. b) System suitability  
143 System suitability test was carried out on freshly prepared solution of GFN -isomer and guaiacol to ensure the  
144 validity of the analytical procedure. Data from six injections were used to confirm system suitability parameters  
145 like retention time, UV-spectra and peak area. The results are presented in Table 2. The values obtained  
146 demonstrated the suitability of the system for the analysis of GFN impurities. The method gives sharp and well  
147 defined peaks with significant RT values which were desired for quantification of GFN related impurities in the  
148 presence of blank, containing GFN, ambroxol hydrochloride and salbutamol sulfate (Table 2).

149 **16 c) Specificity**

150 Specificity is the ability of the method to measure the analytes response in the presence of their potential  
151 impurities and degradation products. Blank (placebo) interference was evaluated by analyzing the blank,  
152 containing GFN, ambroxol hydrochloride, salbutamol sulfate methyl-, propylparaben and citric acid, prepared as  
153 in the test method (Figure ??a). The method showed specificity because GFN -isomer and guaiacol were well-  
154 resolved and no interfering peaks were observed as it appears in Figure ??b. Stress studies were performed either  
155 for guaifenesin impurities and tablet to provide an indication of the stability-indicating property and specificity  
156 of proposed method. The stress conditions employed for degradation study included oxidative hydrolysis and  
157 photochemical degradation. GFN -isomer and guaiacol were found stable under oxidative and photolytic stress  
158 conditions (Figure 3). The peak purity test was carried out for the guaifenesin peak by using the PDA detector  
159 in stress samples. The mass balance (% assay + % sum of all degradants + % sum of all impurities) results  
160 were calculated and found to be more than 95%. The purity of GFN -isomer and guaiacol was unaffected by  
161 the presence of GFN, ambroxol hydrochloride, salbutamol sulfate methyl-, propylparaben and citric acid and  
162 degradation products, and thus confirms the stability-indicating power of the developed method.

163 **17 d) Linearity and LOQ**

164 The linearity was determined by constructing calibration curve. The calibration curves in this study were plotted  
165 between amount of each of analyte versus peak area and the regression equations with a regression coefficient were  
166 obtained. The linear regression data (Table 3) showed good linear relationship over a concentration range of 1-100  
167  $\mu\text{g}/\text{ml}$  for GFN -isomer and 0.1-10.0  $\mu\text{g}/\text{ml}$  for guaiacol. Regression equation for GFN -isomer was  $Y=7.709X +$   
168 0.165 and  $Y=5.588X + 0.005$  for guaiacol with a regression coefficient of 0.9999 for each of analyte. The linearity  
169 of estimated RP-HPLC method was found to be over the concentration range of 1-100  $\mu\text{g}/\text{ml}$  for GFN isomer and  
170 0.1-10.0  $\mu\text{g}/\text{ml}$  for guaiacol which furthermore have been confirmed using back calculation method. The RE %  
171 of linearity back calculation method requirements for analyte calculated to introduced concentration ration to  
172 be less than 15% for at last 6 calibration standards or 75 % of samples, expect LOQ, which should be not less  
173 than 20%. As it shown in the Table 3, the GFN -isomer and guaiacol RP-HPLC assay linearity meets all the  
174 validation quality requirements.

175 **18 e) Accuracy and precision**

176 The intra day precision was determined by measurement of analyte concentration using five replicates of GFN  
177 impurities solutions at three different concentrations 0.5; 5 and 10  $\text{g}/\text{ml}$  for guaiacol and 5,0; 50 and 100  $\text{g}/\text{ml}$   
178 for GFN -isomer two times on the same day and inter day variations were determined similarly on consecutive  
179 days. These concentrations have been selected according to the assay quantification low, medium and high limits  
180 for each of analyte (QCL, QCM and QCH respectively). The repeatability of sample application was assessed 5  
181 times on HPLC followed by recording of the amount of GFN related impurities solutions. The % RSD for peak  
182 values of guaiacol was found to be 2.188% and 2.591% for QCL intra and inter-day precision respectively. The %  
183 RSD and results for GFN related impurities QCL, QCM and QCH concentration are depicted in Table 4, which  
184 reveal intra and inter day variations of analytes concentration.

185 **19 f) Recovery studies**

186 The accuracy of the proposed method was also further assessed by performing recovery experiments using the  
187 standard addition method. Recovery studies of the different samples were carried out for the accuracy parameter.  
188 These studies were carried out at three levels (QCL, QCM and QCH respectively); sample solutions of 5, 50 and  
189 100  $\text{g}/\text{ml}$  as well as standard solutions were prepared for the GFN -isomer and recovery studies were performed  
190 using five replicates. For guaiacol accuracy parameter studies three concentration levels either of sample solutions  
191 as well as standard solutions QCL, QCM and QCH, corresponding to 0.5, 5.0 and 10.0  $\text{g}/\text{ml}$  concentration  
192 respectively were used. The repeatability of sample application was assessed 5 times on HPLC followed by  
193 recording of the peak area of GFN related impurities solutions. Percentage recovery was found to be within the  
194 limits as listed in Table 5.

195 **20 g) Robustness**

196 To determine the robustness of the developed method, experimental conditions were deliberately altered and the  
197 relative retention time of -isomer and guaiacol with respect to guaifenesin; and system suitability parameters  
198 for guaifenesin standard was recorded. The variables evaluated in the study were pH of the mobile phase buffer  
199 ( $\pm 0.2$ ), column temperature ( $\pm 5^\circ\text{C}$ ). In all the deliberate varied chromatographic conditions, all analytes were  
200 adequately resolved and the elution order remained unchanged.

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201 **21 IV.**

202 **22 Conclusion**

203 A new, accurate and selective HPLC method were proposed for the determination of guaifenesin impurities,  
204 2-(2-methoxyphenoxy)-propane-1,3-diol (isomer) and 2-methoxyphenol (guaiacol) in the presence of guaifenesin,  
205 ambroxol hydrochloride , salbutamol sulfate in multi drug components pharmaceutical formulations as per the  
206 ICH guidelines. The methods were found to be simple, selective, precise and accurate. Therefore, these methods  
207 can be used as routine testing as well as stability analysis of guaifenesin and ambroxol impurities in bulk and in  
208 formulations.

209 **23 B**

210 In order to determine the quantification limit analytes concentration in the lower part of calibration curve was  
211 used. GFN -isomer and guaiacol solutions of 1 g/ml and 0.1  $\mu$ g/ml respectively were prepared and analyzed  
212 using six replicates and the amount of each analyte peak area was determined. The LOQ values for GFN -isomer  
213 and guaiacol are shown in Table 3.

214 difference and recovery %) were within the acceptance criteria.



1

Figure 1: Figure 1 :

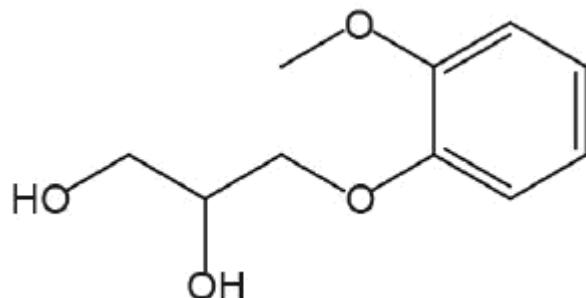


Figure 2:

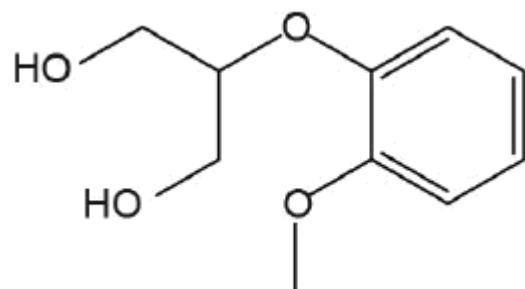


Figure 3:

215

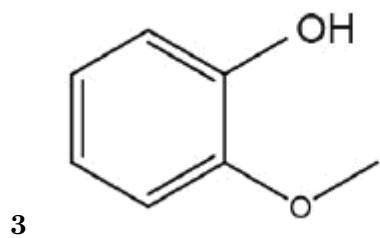


Figure 4: \*Figure 3 :

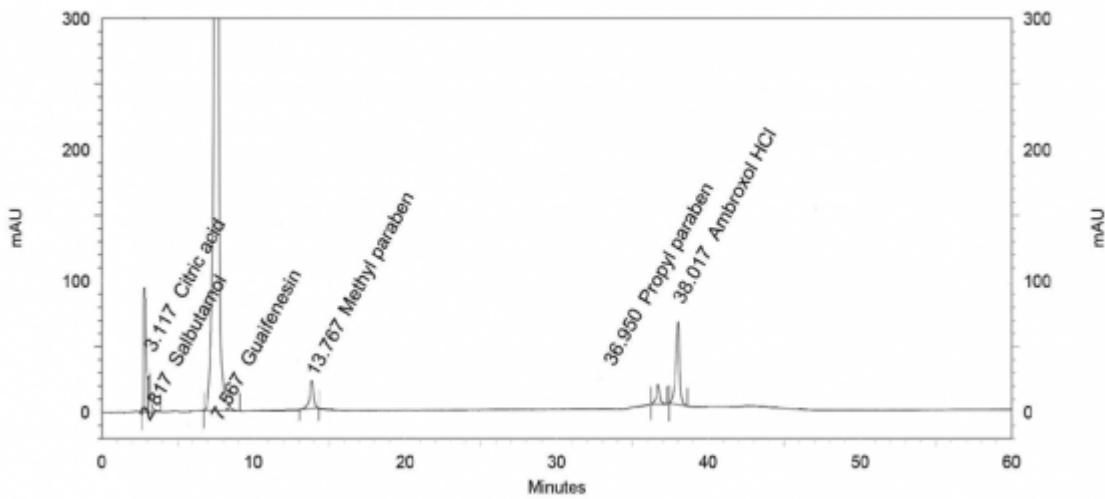


Figure 5:

1

## ambroxol impurities

Parameter/Condition	Specification			
Column				
Mobile phase gradient	Solvent A-0.1 M ammonium acetate buffer of pH 6.8 Solvent B -acetonitrile			
Working wavelength	275 nm			
Column temperature	45 o C			
Sample volume	10 uL			
Run time	60 min			
Time	Flow	Comp A	Comp B	
(min)	(ml/min)	(%)	(%)	
1	0.0	1.0	25	75

2

Gradient elution

Figure 6: Table 1 :

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## 2

Parameters		GFN ?-isomer
R T	12,726 $\pm$ 0,0087	6,318 $\pm$ 0,060
Peak area	17517,4 $\pm$ 417,17	11591,6 $\pm$ 180,76
R T %RSD ¶	0,068	0,95
Peak area %RSD ¶	2,381	1,559

Figure 7: Table 2 :

## 3

Parameters	method	
	guaiacol	GFN ?-isomer
Concentration range	0.1-10 µg/ml	1-100 µg/ml
Slope	5.588	7.709
Intercept	0.005	0.165
Correlation coefficient	0.9999	0.9999
Regression equation	$Y=5.588X + 0.005$	$Y=7.709X + 0.165$
RE%*	-2.051	0.175
LOQ (µg/ml)	0.098 $\pm$ 0.0029	1.017 $\pm$ 0.0109
LOQ %RSD	2.974	1.078

[Note: \* RE % of linearity back calculation method represented the percentage of ration  $100 \times (E-T)/T$ , where E is a calculated concentration and T -is a introduced concentration of the analyte. All data represent Mean SD for n=6 standard samples for each of mentioned analyte. Grubbs test detects no outliers from normal distribution (? = 0.02). %RSD =  $100 \times (SD/Mean)$ .]

Figure 8: Table 3 :

## 4

Parameters

[Note: \*]

Figure 9: Table 4 :

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Parameters	QCL 0.5 ?g/ml	QCM 5 ?g/ml	QCH 10 ?g/ml	QCL 5 ?g/ml	QCM 50 ?g/ml	GFN ?-isomer QCH 100 ?g/ml
Peak area of	8697 ±	87717±	180311	60141	624380 ±	1369669
sample*			±	±	±	±
	249.2	832.2	2290	780.7	18210	44541

Figure 10: Table 5 :

### **216 .1 Acknowledgements**

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