CHAPTER 6

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PARACETAMOL, TRAMADOL HYDROCHLORIDE AND DOMPERIDONE IN A COMBINED DOSAGE FORM

6.1 INTRODUCTION OF DOSAGE FORM AND LITERATURE REVIEW

Paracetamol has been in use as an analgesic for more than 30 years and is accepted as a very effective treatment for pain relief and fever in adults and children (Sweetman 2009). Tramadol hydrochloride is an opioid analgesic. It also has noradrenergic and serotonergic properties that may contribute to its analgesic activity and is used for moderate-to-severe pain (Sweetman 2009). Domperidone is an antidopaminergic drug that is generally used orally, rectally or intravenously to suppress nausea and vomiting (Sweetman 2009). Chemical structures of Paracetamol (Chemically: N-(4-hydroxyphenyl) acetamide), tramadol (Chemically: (1RS, 2RS)-2-[(Dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol) and domperidone (Chemically: 5-chloro-1-(1-[3-(2-oxo-2, 3-dihydro-1H-benzoimidazol-1-yl) propylpiperidin-4-yl])-1H-benzoimidazol-2(3H)-one) are shown in Figures 6.1 to 6.3.
Figure 6.1 Chemical structure of paracetamol
(MF: C₈H₉NO₂, MW: 151)

Figure 6.2 Chemical structure of tramadol
(MF: C₁₆H₂₅NO₂, MW: 263)

Figure 6.3 Chemical structure of domperidone
(MF: C₂₂H₂₄ClN₅O₂, MW: 426)
Chromatographic methods for the determination of paracetamol, tramadol hydrochloride and domperidone as single entities are included in BP. A survey of the literature revealed that very few methods have been reported for the determination of paracetamol and tramadol hydrochloride or paracetamol and domperidone as combined drug formulations (Betal et al 2009, Ghorbani-Bidkorbbeh et al 2010, Kapil et al 2009, Karthick et al 2007, Srinivasan et al 2007, Yadav 2009 and Zhu et al 2007). The literature also shows that assay methods for one of these drugs in combination with other drugs have been reported (Patel et al 2008 and Salmeron-Garcia et al 2009). Recently, Birajdar et al (2009) reported the assay of paracetamol and tramadol hydrochloride in a combination drug product in which domperidone was used as an internal standard. A method, which utilised UV-spectrophotometry for the simultaneous determination of paracetamol, tramadol hydrochloride and domperidone, has been published (Sawant et al 2010). Jain et al (2010) published the HPLC method for the simultaneous determination of paracetamol, tramadol hydrochloride and domperidone, which showed peak tailing of paracetamol. Moreover, tramadol hydrochloride peak eluted in the void of paracetamol peak, as the peak area of tramadol hydrochloride was very low; setting the integration parameter in the HPLC system became difficult. This quantification led to a bias result. In view of these flaws, the reported method is not suitable for analysis in the pharmaceutical industry.

6.1.1 Target of the Work

To the best of our knowledge, no work utilising an isocratic and stability-indicating RP-HPLC method has been applied for the simultaneous estimation of paracetamol, tramadol hydrochloride and domperidone. This paper describes the development and validation of a precise, specific and reliable HPLC method with isocratic elution for the simultaneous determination of paracetamol, tramadol hydrochloride and domperidone in pharmaceutical formulations.
6.2 EXPERIMENTAL

6.2.1 Materials and Reagents

Pharmaceutical grade paracetamol, tramadol hydrochloride and domperidone were obtained as a gift from M/S GS Lab (Baddi, India). A commercial combination drug product was tested. Each tablet contained 325 mg of paracetamol, 37.5 mg of tramadol hydrochloride and 10 mg of domperidone. HPLC grade acetonitrile and methanol were purchased from Merck (India). Buffer materials and all other chemicals used were of analytical reagent grade. High purity water was generated from a Millipore Milli-Q plus purification system.

6.2.2 Instrumentation

The HPLC system was a Waters 2695 binary pump plus auto sampler and a 2996 photo diode array as well as 2487 UV detector (Waters Corporation, Milford, USA) was used for all method development and validation.

6.2.3 Preparation of Standard Solution

A mixture of tramadol hydrochloride and domperidone standard stock solution was prepared by transferring 37.5 mg of tramadol hydrochloride and 10.0 mg of domperidone into a 100 ml volumetric flask. A 50 mL portion of diluent (mobile phase) was added, sonicated to dissolve and cooled to room temperature. The solution was made up with diluent and mixed.

Paracetamol (16.5 mg) was accurately weighed and transferred into a 50 mL volumetric flask and 20 mL of diluent (mobile phase) was added to
dissolve it. Further, a 5 mL portion of tramadol hydrochloride and domperidone standard stock solution was added and the volume made up with diluent to obtain a solution containing 0.33 mg/mL of paracetamol, 0.0375 mg/mL of tramadol hydrochloride and 0.01 mg/mL of domperidone. The solution was mixed and filtered through a 0.45 μm nylon syringe filter and 10 μL was injected.

6.2.4 Sample Preparation

Five tablets were weighed and finely powdered with a mortar and pestle and transferred into a 500 mL volumetric flask (The mortar and pestle was thoroughly washed with diluent and the washed solution was transferred into a 500 mL volumetric flask). Approximately 200 mL of diluent was added to the flask and the contents were sonicated for 30 min. The volume was made up to 500 mL with more diluent and mixed thoroughly. After that, 5 mL of the resulting solution was diluted to 50 mL with diluent to give concentrations of 0.33 mg/mL, 0.0375 mg/mL and 0.01 mg/mL for paracetamol, tramadol hydrochloride and domperidone respectively. The resulting solution was filtered using a 0.45 μm nylon syringe filter; 10 μL of the resulting solution was injected for analysis.

6.3 RESULTS AND DISCUSSION

6.3.1 Optimization of Chromatographic Method

To develop a suitable and isocratic RP-HPLC method for the simultaneous determination of paracetamol, tramadol hydrochloride and domperidone, different buffer pH and column chemistries were applied to achieve the separation of all three components. The main objective of the chromatographic method was to develop a single method for all three components. In the case of domperidone, the dose is very low compared to paracetamol. Finally, the mobile phase consisting of 0.1 % trifluoroacetic acid,
acetonitrile and methanol in the ratio of 70:25:5 (v/v) at a flow rate of 1.0 mL/min using Peerless basic C$_{18}$ (250 mm x 4.6 mm, 5 micron) column was found to be suitable, allowing for good separation of paracetamol, tramadol and domperidone.

In the optimized chromatographic conditions, the peak shape of paracetamol, tramadol and domperidone was symmetrical with a satisfactory resolution. The main system suitability parameter of theoretical plates for paracetamol was more compared to recently published methods. This indicates that the paracetamol had very good retention in the optimized condition and the resolution between paracetamol and tramadol was > 15. The response of paracetamol, tramadol and domperidone was adequate at 272 nm. To prove the specificity of the method, all individual component and diluent solutions were injected. No blank interference was found in the compounds (Figures 6.4 to 6.7). The optimized method is shown in Table 6.1.

![Figure 6.4 Individual injection of paracetamol](image-url)

**Figure 6.4** Individual injection of paracetamol
Figure 6.5  Individual injection of tramadol

Figure 6.6  Individual injection of domperidone

Figure 6.7  HPLC overlay chromatogram of blank (A) and standard solution (B)
Table 6.1  Optimized chromatographic method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Mixture of 0.1 % of trifluoroacetic acid, acetonitrile and methanol in the ratio 70:25:5 (v/v)</td>
</tr>
<tr>
<td>Diluent</td>
<td>Mobile phase</td>
</tr>
<tr>
<td>Column</td>
<td>Peerless basic C\textsubscript{18}, 250 mm x 4.6 mm, 5 micron</td>
</tr>
<tr>
<td>Column oven temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>272 nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
</tr>
</tbody>
</table>

6.3.2 Method Validation

The developed HPLC method was validated according to ICH and FDA guidelines in terms of precision, ruggedness, linearity, specificity, selectivity, robustness and accuracy.

6.3.2.1 System Suitability

To check the system and column performance, the standard solution was injected five times and few parameters were monitored. These parameters were the tailing factor (< 2.0), theoretical plates (> 8000 for paracetamol, not less than 12000 for tramadol and > 15000 for domperidone) and % RSD of paracetamol, tramadol and domperidone (< 2 %). System suitability results are shown in Table 6.2.
Table 6.2  System suitability results

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>USP tailing factor</th>
<th>Theoretical plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>3.87</td>
<td>1.2</td>
<td>11392</td>
</tr>
<tr>
<td>Tramadol</td>
<td>9.85</td>
<td>1.2</td>
<td>16724</td>
</tr>
<tr>
<td>Domperidone</td>
<td>22.40</td>
<td>1.1</td>
<td>18703</td>
</tr>
</tbody>
</table>

6.3.2.2  Precision

Assay method precision was assessed using six independent test solutions. The intermediate precision of the assay method was also evaluated using different analysts, columns and HPLC systems on different days.

Percentage RSD values of paracetamol, tramadol hydrochloride and domperidone were 1.1 %, 0.9 % and 1.3 % respectively in method precision and 0.8 %, 0.5 % and 1.2 % respectively in intermediate precision. The low RSD values (< 2 %) showed the suitability of the method for the determination of paracetamol, tramadol hydrochloride and domperidone in a combination tablet formulation. Precision results are shown in Tables 6.3 and 6.4.
### Table 6.3  Method precision results

<table>
<thead>
<tr>
<th>Injection</th>
<th>Paracetamol (%)</th>
<th>Tramadol hydrochloride (%)</th>
<th>Domperidone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101.2</td>
<td>97.3</td>
<td>98.1</td>
</tr>
<tr>
<td>2</td>
<td>99.3</td>
<td>98.1</td>
<td>96.9</td>
</tr>
<tr>
<td>3</td>
<td>100.2</td>
<td>97.2</td>
<td>98.2</td>
</tr>
<tr>
<td>4</td>
<td>98.5</td>
<td>99.4</td>
<td>97.2</td>
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<tr>
<td>5</td>
<td>98.8</td>
<td>98.5</td>
<td>100.3</td>
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<tr>
<td>6</td>
<td>100.5</td>
<td>97.5</td>
<td>99.1</td>
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<tr>
<td>Average</td>
<td>99.8</td>
<td>98.0</td>
<td>98.3</td>
</tr>
<tr>
<td>SD</td>
<td>1.05</td>
<td>0.85</td>
<td>1.25</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.1</td>
<td>0.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>

### Table 6.4  Intermediate precision results

<table>
<thead>
<tr>
<th>Injection</th>
<th>Paracetamol (%)</th>
<th>Tramadol hydrochloride (%)</th>
<th>Domperidone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.1</td>
<td>98.6</td>
<td>98.6</td>
</tr>
<tr>
<td>2</td>
<td>99.2</td>
<td>98.5</td>
<td>97.2</td>
</tr>
<tr>
<td>3</td>
<td>98.3</td>
<td>98.9</td>
<td>98.9</td>
</tr>
<tr>
<td>4</td>
<td>98.1</td>
<td>99.1</td>
<td>100.3</td>
</tr>
<tr>
<td>5</td>
<td>97.9</td>
<td>98.9</td>
<td>100.1</td>
</tr>
<tr>
<td>6</td>
<td>99.9</td>
<td>99.8</td>
<td>100.3</td>
</tr>
<tr>
<td>Average</td>
<td>98.8</td>
<td>99.0</td>
<td>99.2</td>
</tr>
<tr>
<td>SD</td>
<td>0.77</td>
<td>0.46</td>
<td>1.24</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.8</td>
<td>0.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>
6.3.2.3 Linearity

Peak areas versus concentrations were plotted for paracetamol, tramadol hydrochloride and domperidone at five different concentration ranges between 50 % and 150 % of target level. Peak areas versus concentrations were plotted for paracetamol, tramadol hydrochloride and domperidone at five different concentration ranges between 50 % and 150 % of target level. Paracetamol, tramadol hydrochloride and domperidone showed linearity in the range of 165-495 µg/mL, 18.75-56.25 µg/mL and 5-15 µg/mL respectively. Linearity plots are shown in Figures 6.8 to 6.10. The results obtained are represented by the following linear regression equations:

\[ Y_{\text{Paracetamol}} = 71017x - 48000 \ (r^2=0.9991) \]
\[ Y_{\text{Tramadol hydrochloride}} = 1149.9x +500.2 \ (r^2=0.9998) \]
\[ Y_{\text{Domperidone}} = 574.21x - 460.2 \ (r^2=0.9993) \]

![Linearity plot for paracetamol](image)

Figure 6.8 Linearity plot for paracetamol
6.3.2.4 Specificity and Selectivity

Stress studies of the drug product are utilised for the identification of possible degradation products and the validation of the stability-indicating analytical procedure. It is the ability of the analytical method to measure the analyte response in the presence of its degradation products and sample matrix.
Forced degradation studies were performed on the tablet samples using the following conditions: acid hydrolysis (0.1 N HCl, 8 hours), base hydrolysis (0.1 N NaOH, 8 hours), oxidation (3 % H2O2, 8 hours), heat (80°C for 48 hours) and photolysis (UV 254nm, 48 hours). All these samples were appropriately diluted with diluent and injected into the HPLC. Peak purity test was carried out for paracetamol, tramadol and domperidone using a PDA detector in the stress samples.

The results obtained from forced degradation study are summarised in Table 6.5. In acidic conditions, the degradation product was formed at a retention time (RT) of 8.115 min. In a basic condition, the degradation product formed was at an RT of 4.557 min which had > 3.0 resolutions from paracetamol. In a peroxide condition, the degradation products formed were at RTs of 3.284 min, 4.175 min and 6.116 min; the close eluting impurity at 4.175 min had > 1.3 resolutions from paracetamol. No degradation occurred in heat and photolysis-stressed samples. In all degradation samples, purity angles were less than threshold, indicating the purity of the peaks and stability-indicating nature of the developed HPLC method. Degradation sample chromatograms are shown in Figure 6.11.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Paracetamol (%)</th>
<th>Tramadol hydrochloride (%)</th>
<th>Domperidone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sample</td>
<td>99.5</td>
<td>99.3</td>
<td>99.6</td>
</tr>
<tr>
<td>Acid hydrolysis</td>
<td>97.5</td>
<td>98.2</td>
<td>98.5</td>
</tr>
<tr>
<td>Base hydrolysis</td>
<td>96.2</td>
<td>96.5</td>
<td>99.2</td>
</tr>
<tr>
<td>Peroxide oxidation</td>
<td>92.3</td>
<td>98.2</td>
<td>97.5</td>
</tr>
<tr>
<td>Heat (80°C)</td>
<td>98.9</td>
<td>98.9</td>
<td>99.2</td>
</tr>
<tr>
<td>Light (254 nm)</td>
<td>99.1</td>
<td>99.1</td>
<td>99.3</td>
</tr>
</tbody>
</table>
Figure 6.11 Typical LC chromatogram of normal, acid, base and peroxide sample
6.3.2.5 Accuracy

The accuracy of the method was determined for paracetamol, tramadol hydrochloride and domperidone by recovery experiments. Known amounts of paracetamol, tramadol hydrochloride and domperidone bulk sample (of the specified limits) at levels of 80 %, 90 %, 100 %, 110 % and 120 % respectively were taken for analysis, in a triplicate. The results for accuracy of the method are given in Table 6.6.

Table 6.6 Recovery study results

<table>
<thead>
<tr>
<th>Compound</th>
<th>Level (%)</th>
<th>Amount added (µg/mL)</th>
<th>Recovery (%)</th>
<th>% RSD (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>80</td>
<td>264</td>
<td>100.4</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>297</td>
<td>98.4</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>330</td>
<td>99.7</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>363</td>
<td>99.1</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>396</td>
<td>99.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Tramadol hydrochloride</td>
<td>80</td>
<td>30</td>
<td>99.9</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>33.75</td>
<td>99.22</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>37.5</td>
<td>100.3</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>41.25</td>
<td>98.31</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>45</td>
<td>99.6</td>
<td>1.86</td>
</tr>
<tr>
<td>Domperidone</td>
<td>80</td>
<td>8</td>
<td>99.3</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>9</td>
<td>99.1</td>
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<td></td>
<td>100</td>
<td>10</td>
<td>99.5</td>
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<td></td>
<td>110</td>
<td>11</td>
<td>100.2</td>
<td>1.22</td>
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<tr>
<td></td>
<td>120</td>
<td>12</td>
<td>99.8</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Recoveries of paracetamol, tramadol hydrochloride and domperidone in bulk drug samples were between 98.0% and 102.0%, indicating good accuracy of the developed method.

6.3.2.6 Robustness

Robustness was established by analyzing the system suitability standard (n = 5) and sample (n = 3) at 25°C and 35°C (nominal = 30°C), at flow rates of 0.9 mL/min and 1.1 mL/min of the nominal flow (i.e., 1.0 mL/min) and ±10% change of buffer, acetonitrile and methanol (nominal ratio = 70:25:5, v/v). The tailing factors and theoretical plates of paracetamol, tramadol and domperidone were evaluated. The tailing factor of all the three peaks was less than 1.3 in all tested conditions and the theoretical plates of the peaks were found in the ±10% range from the original values. In all the deliberate changes, no significant change of assay value was observed. However, the current method was robust for such deliberate changes. Percentage RSD of the standard was < 1.0 and the assay data of the drug components were in the range of 98% to 102%, indicating robustness of the method. Robustness observations are shown in Table 6.7.
### Table 6.7 Robustness study results

<table>
<thead>
<tr>
<th>Variations</th>
<th>Paracetamol (%)</th>
<th>Tramadol hydrochloride (%)</th>
<th>Domperidone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mL/min (original)</td>
<td>98.1</td>
<td>99.2</td>
<td>98.3</td>
</tr>
<tr>
<td>0.9 mL/min</td>
<td>97.3</td>
<td>99.0</td>
<td>98.1</td>
</tr>
<tr>
<td>1.1 mL/min</td>
<td>98.5</td>
<td>98.8</td>
<td>99.1</td>
</tr>
<tr>
<td><strong>Organic composition</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 %, v/v (original)</td>
<td>98.1</td>
<td>99.2</td>
<td>98.3</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(22.5 %, v/v)</td>
<td>98.3</td>
<td>99.2</td>
<td>98.0</td>
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<tr>
<td>Acetonitrile</td>
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</tr>
<tr>
<td>(27.5 %, v/v)</td>
<td>98.9</td>
<td>99.0</td>
<td>98.9</td>
</tr>
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<td><strong>Organic composition</strong></td>
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</tr>
<tr>
<td>Methanol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 %, v/v (original)</td>
<td>98.1</td>
<td>99.2</td>
<td>98.3</td>
</tr>
<tr>
<td>Methanol</td>
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</tr>
<tr>
<td>(4.5 %, v/v)</td>
<td>99.0</td>
<td>99.9</td>
<td>97.9</td>
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<td>Methanol</td>
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<td>(5.5 %, v/v)</td>
<td>98.7</td>
<td>99.5</td>
<td>98.0</td>
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<td>99.2</td>
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<td>35°C</td>
<td>97.8</td>
<td>98.5</td>
<td>97.9</td>
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</table>
6.3.2.7  Application of the Developed Method

The ability of the optimized method to determine paracetamol, tramadol hydrochloride and domperidone simultaneously in a commercial drug product was tested using six individual preparations. The combination tablet, containing 325 mg of paracetamol, 37.5 mg of tramadol hydrochloride and 10 mg of domperidone, was utilised for the present study (Decotram-PD®, manufactured Worth Medicine, Chandigarh, India). The assay results for paracetamol, tramadol hydrochloride and domperidone were 99.2 %, 98.9 % and 100.2 % respectively. The assay values were close to the labeled claim for all the three drugs, indicating that interference by excipients was insignificant. The low values of RSD (< 2 %) for the assay established the precision of the proposed method.

6.3.2.8  Conclusion

The developed isocratic HPLC method for the simultaneous determination of paracetamol, tramadol hydrochloride and domperidone in a pharmaceutical dosage form is specific, precise, accurate, linear and robust. An excellent correlation exists between peak area and concentration for the three drugs. The developed method is a stability-indicating method and can be conveniently used by quality control outfits to determine the contents of paracetamol, tramadol hydrochloride and domperidone simultaneously in routine samples.