LC-MS/MS determination of urinary 5-hydroxytryptophol glucuronide and 5-hydroxyindole-3-acetic acid

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AIMS: The serotonin metabolites 5-hydroxytryptophol glucuronide (GTOL) and 5-hydroxyindole-3-acetic acid (5-HIAA) are normal constituents in human urine. Following alcohol drinking, the proportion of these metabolites is shifted towards GTOL, and this can be used as a biomarker. The aim of this study was to develop an LC-MS/MS method for GTOL and 5-HIAA analysis in urine suitable for clinical application.

METHODS: Urine patient samples were diluted to 1:1 with deionised water containing deuterium-labelled internal standards. The atmospheric pressure ionization (APCI) interface used with the mass spectrometer was operating in the negative mode. Analysis was performed by the single reaction monitoring (SRM), using the precursor ion at m/z 352 and the product ion at m/z 176 for GTOL, and precursor ion at m/z 190 and product ion at m/z 144 for 5-HIAA. Diluted sample of 15 µl was injected on a Hypercarb column (100 mm × 2.1, 5 µm) equipped with Hypercarb guard cartridge (10 mm × 2.1, 5 µm). The mobile phase consisted of 20 mmol/L ammonium acetate (pH 7.0) with 10 and 60% acetonitrile using gradient elution at a flow rate of 300 µL/min. The total run time was 12 min. The GC-MS method for 5-HTOL had a measuring range up to 10 µmol/L and the intra- and inter-assay coefficients of variation were less than 7%. The HPLC method with electrochemical detection for 5-HIAA had linear response in the range of 0-65 µmol/L, and the intra-and interassay coefficients of variation were about 5 and 7%, respectively.

RESULTS: Linear response curves were obtained between 0.017 and 90.0 µmol/L for GTOL, respectively 0.11 and 66.4 µmol/L for 5-HIAA. The LOD (s/n ~ 3:1) were estimated 0.005 µmol/L for GTOL, respectively 0.03 µmol/L for 5-HIAA. The LOQ (s/n ~ 10:1) were 0.017 µmol/L for GTOL, respectively 0.11 µmol/L for 5-HIAA The capacity factor for the first eluting compound GTOL was 4.1 and no ion suppression occured. The comparison of the present LC-MS/MS method for GTOL and GC-MS method for 5-HTOL (free plus liberated from GTOL) respectively LC-MS/MS method and HPLC with electrochemical detection for 5-HIAA included a total of 47 patient urine samples. The method correlations for GTOL and 5-HIAA were respectively 0.9994 and 0.9998. The mean ratio for GC-MS (5-HTOL) over LC-MS/MS (GTOL) for all samples, covering the range 0.072 – 2.20 µmol/L, was 1.05.
The intra- and inter-assay CV for GTOL were 2.9 and 3.5% respectively at 0.524 µmol/L, and 1.9 and 2.0% respectively at 0.058 µmol/L. The intra- and inter-assay CV for 5-HIAA were 6.0 and 8.1% respectively at 10.4 µmol/L, and 2.6 and 2.7% respectively at 2.87 µmol/L.

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CONCLUSIONS:

- Direct analysis of GTOL and 5-HIAA in urine by APCI-LC-MS/MS is a technique with advantages over GC-MS and solid-phase extraction LC-MS analysis because of higher sample throughput and shorter analysis time, no need for solvent extraction and derivatization as in GC-MS.
- Simultaneous analysis of both alcohol markers in the same run is very convenient because the ratio GTOL/5-HIAA can be used to detect alcohol drinking.
- The LC-MS/MS method for GTOL developed in this study correlates well with previous GC-MS methods and provides the possibility to use the main metabolite GTOL as an alcohol marker in clinical and forensic purposes.
- The LC-MS/MS method for 5-HIAA developed in this study correlates well with previous HPLC method.
- The LC-MS/MS method may represent a major improvement for routine clinical application by its simplicity. The product scan spectrum for both compounds provides the possibility to choose even more transitions which can be used as qualifiers.

KEYWORDS: *LC-MS/MS, 5-hydroxytryptophol, Alcohol marker*

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