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Blood-brain barrier specific permeability assay reveals *N*-methylated tyramine derivatives in standardised leaf extracts and herbal products of *Ginkgo biloba*

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ABSTRACT

The linkage between the central nervous system availability and neuropharmacological activity of the constituents of *Ginkgo biloba* L. extracts (GBE) is still incomplete. In this study, the in vitro blood-brain barrier (BBB) permeability profile of the standardised GBE was investigated by the parallel artificial membrane permeability assay (PAMPA). Biomarkers, such as terpene trilactones, flavonoid aglycones and ginkgotoxin exerted moderate or good BBB-permeability potential (BBB+), while glycosides and biflavones were predicted as unable to pass the BBB. *N*-methyltyramine (NMT) and *N,N*-dimethyltyramine or hordenine (Hor) were identified among BBB+ compounds, while subsequent direct HRMS analysis revealed tyramine (Tyr) and *N,N,N*-trimethyltyramine or candicine (Can) in GBE as trace constituents. Distribution of Tyr, NMT, Hor and Can was determined by a validated ion-exchange mechanism-based liquid chromatography–electrospray ionisation–mass spectrometry (LC-ESI–MS) method in *G. biloba* samples, such as herbal drugs and dietary supplements. The total content of the four tyramine derivatives in various GBEs ranged from 7.3 up to 6357 μg/g dry extract with NMT and Hor as most abundant ones. Considering the pharmacological activities and the revealed fluctuation in the concentration of the analysed adrenergic protoalkaloids, the presented rapid LC-ESI–MS method is proposed for monitoring of the levels of Tyr, NMT, Hor and Can in *G. biloba* products.

1. Introduction

Ginkgo biloba L. (Ginkgoaceae) has become one of the most popular and most studied medicinal plants used in central nervous system (CNS) related disorders, such as early dementia [1–4]. The beneficial cerebrovascular and neuroprotective effects of the standardised *G. biloba* leaf extracts (GBE; e.g., EGb761®) have been documented in several preclinical and clinical studies [5,6]. The neurobiological activity of GBE is primarily attributed to two major groups of constituents: the flavonol glycosides and the unique terpene trilactones [7]. Accordingly, CNS-related pharmacokinetic investigations on GBEs focused exclusively on the characterisation of the following ingredients: ginkgolides (A, B, C, and J), bilobalide, quercetin, kaempferol, and isorhamnetin, respectively. These components, referred to as ginkgo biomarkers, however, make up only approx. 30% (w/w) of standardised GBEs [2], furthermore their reported brain exposure data indicate low or moderate CNS-bioavailability [8]. As a consequence, the linkage between the neuropharmacological activity and the pharmacokinetic profile of GBE is still not completely revealed [9].

To effectively act on the CNS, compounds in plant extracts, such as in GBE must cross the blood-brain barrier (BBB) first. The exact in vivo study of the BBB-transport of multi-component plant extracts, however, is practically not feasible and limited typically to the investigation of selected biomarkers. The BBB-specific parallel artificial membrane permeability assay (PAMPA), developed by Di et al. [10], and optimized by our group [11], could represent a reasonable in vitro approach for this problem. We have shown in our previous work, that the PAMPA-BBB system is capable to model simultaneously the rate of transcellular passive diffusion of

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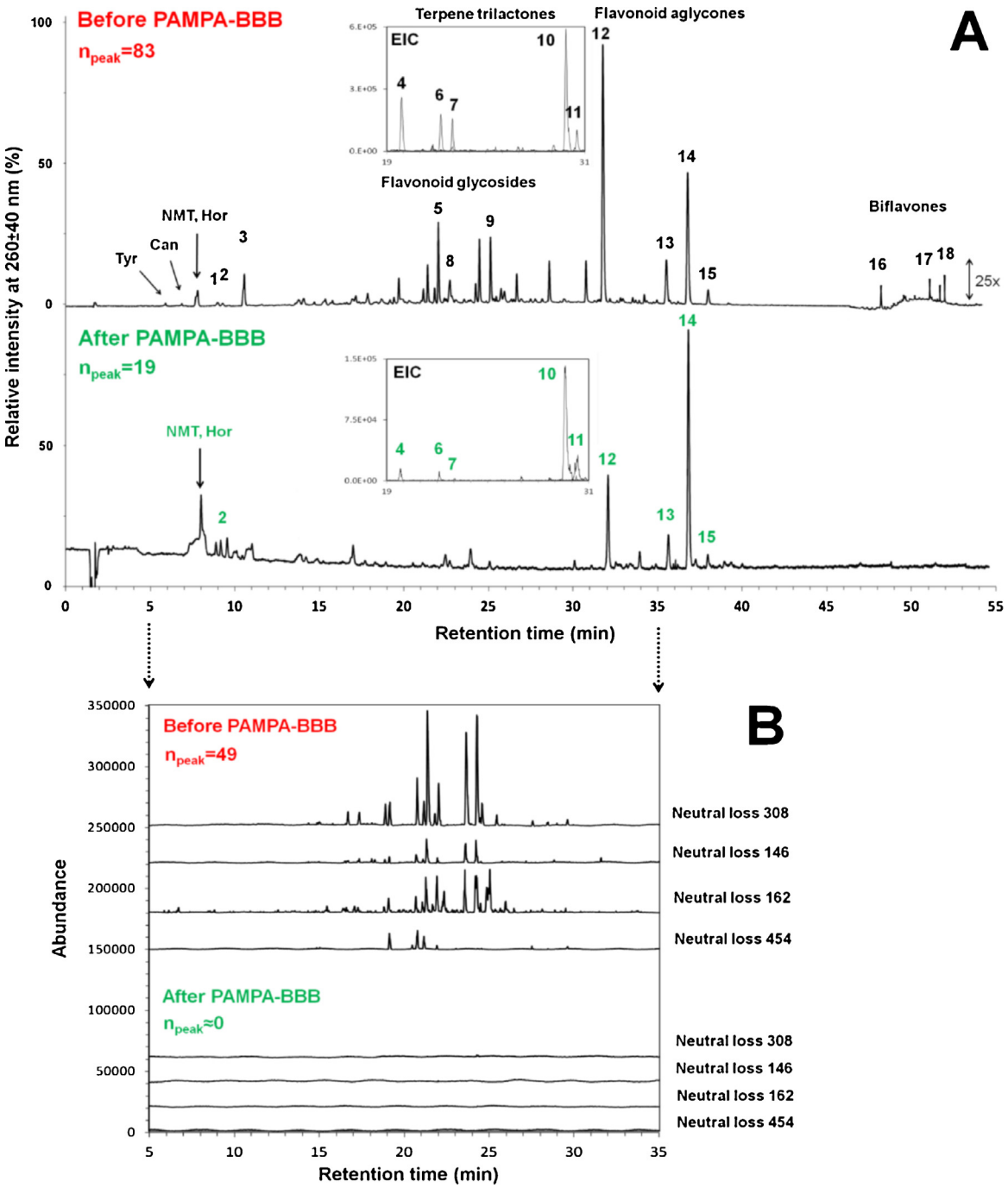

Fig. 1. LC–MS chromatograms obtained in the PAMPA-BBB study of a standard *G. biloba* extract (standard A). (A) UV trace at 260 ± 40 nm of the stock solution (10 mg/mL in MeOH) before (top) and acceptor solution after (bottom) the PAMPA-BBB experiment. BBB+ compounds marked in green. For five terpene trilactones (**4**, **6**, **7**, **10**, **11**) extracted ion chromatograms (EIC) are presented. (B) Neutral loss scan chromatograms at 146, 162, 308, and 454 Da of the same samples. For peak identities see Table 1.

single molecules in complex mixtures across the BBB by measuring the effective permeability (P_e, cm/s) of an artificial lipid membrane [12]. Thus, by mean of this non-cell-based permeation test, BBB-permeability potential of each constituent in an extract could be predicted in a simple and robust way. For instance, brain penetrability of the anti-migraine sesquiterpene lactone parthenolide [12], the prolyl oligopeptidase inhibitor flavonoid baicalin [13], and the acetylcholinesterase inhibitor alkaloid undulatine [14] were characterised using this approach. In addition, since the PAMPA assay functions practically as an unique physicochemical filtering tool, efficiency of the dereplication of compounds with high BBB-permeability (denoted as BBB+) is significantly increased by the direct LC–MS and NMR analysis of the PAMPA-filtered samples (i.e., solution in the acceptor compartment). These features of the PAMPA-BBB assay motivated us to investigate the in vitro BBB-permeability profile of GBE by a PAMPA-BBB/LC–MS assay.

In the first part of this study, the European Pharmacopoeia reference standard of GBE was thoroughly analysed by a PAMPA-BBB/LC–MS assay. Biomarkers in GBE were resolved and identified in an optimized LC–MS method, and their BBB-permeability potential was characterised in a comprehensive fashion. To selectively detect abundant glycoside-type compounds, neutral loss scan chromatograms for mono-, di-, and triglycosides were acquired. Surprisingly, *N*-methylated derivatives of tyramine were discov-