PharmacoLearn

MPG103T Phytochemistry, M.Pharm Semester 1

Unit 4: Phytochemical Fingerprinting

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4.1 The fingerprint idea: characterising a whole extract

Units 1 to 3 deal with single constituents. This unit changes the object: the herbal extract itself, a mixture no single marker assay can describe. A phytochemical fingerprint is the characteristic pattern an extract produces under a defined method: the set of peaks on a chromatogram, with their positions and relative sizes, generated under fixed conditions. Its logic is that of a human fingerprint: the pattern as a whole identifies the material, though no single peak is the identity. A genuine extract of the right species, part and process gives its pattern; an adulterated, substituted or exhausted extract gives a different one; two batches of the same product give patterns alike within defined limits. Fingerprinting therefore answers the three questions a marker assay cannot: is this the right plant and part (identity), is this batch like the approved batch (consistency), and has anything foreign or missing changed the whole composition (adulteration and deterioration). Its working rules follow: the method is fixed in every detail, reference material of verified identity is run beside the sample, and the whole pattern is compared, by profile similarity and the ratios of characteristic peaks, with marker peaks identified inside the pattern where standards exist. The workflow below shows a characterisation built this way.

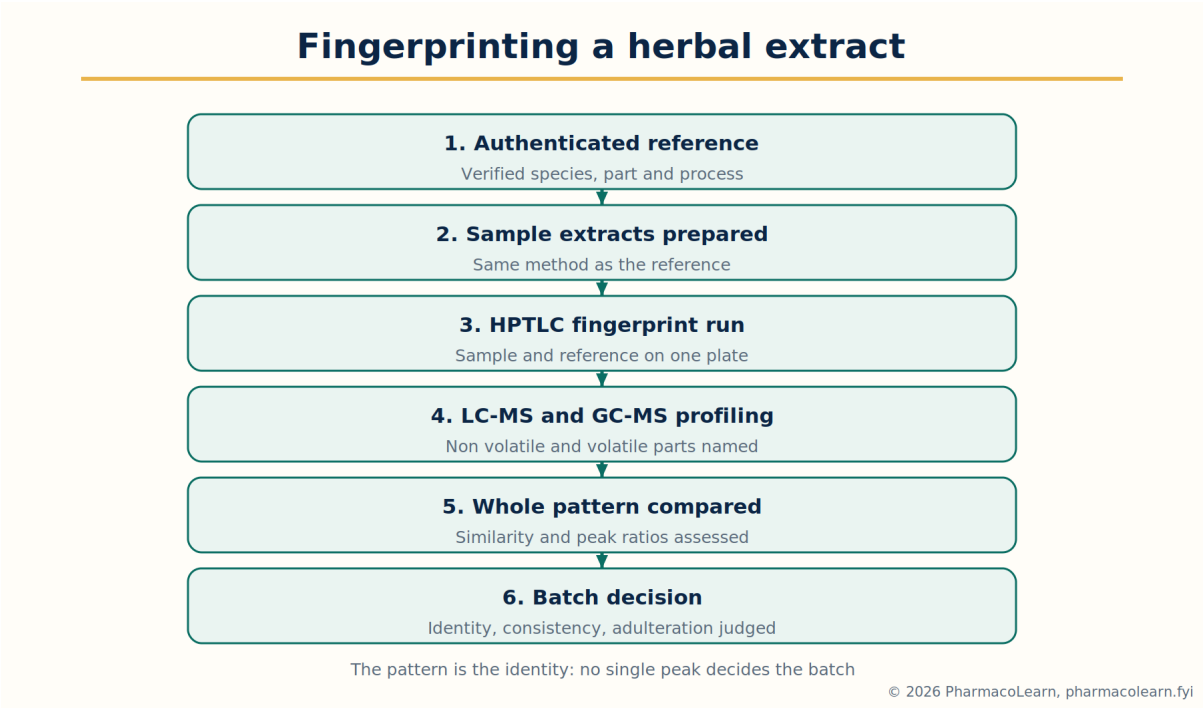

Figure: The fingerprinting workflow. The reference extract and the sample travel through identical conditions, because the comparison is only valid inside one fixed method.

4.2 HPTLC fingerprinting

High performance thin layer chromatography is the fingerprinting method the syllabus names first, and the identity workhorse of herbal quality control. HPTLC uses plates with a fine, uniform layer, most often silica gel, samples applied as narrow bands, development in a controlled chamber, and instrumental scanning of the finished plate, densitometry, under ultraviolet light and after derivatisation where colour forming reagents reveal further zones. One plate carries the sample and the reference standards and reference

extract side by side in the same run under identical conditions, so the comparison the fingerprint idea demands is built into the format. The fingerprint is the sequence of zones at their retention factors, with their colours under ultraviolet light and after spraying: a senna fingerprint shows its sennoside zones against sennoside standards, a liquorice fingerprint its glycyrrhizin zone, and the accompanying zones distinguish the genuine drug from a substitute even where the marker is present. HPTLC also quantifies, the scanner measuring zone intensity against standards on the same plate, so one run delivers identity, fingerprint comparison and marker assay together. Its limits are moderate resolution against HPLC and sensitivity to chamber conditions, which is why saturated chambers, fixed development distance and system suitability checks are part of the method.

4.3 LC-MS in the characterisation of herbal extracts

Liquid chromatography with mass spectrometry joins HPLC separation to mass spectrometric identification, and in extract characterisation it does three jobs no optical detector can do. First, identification by mass: each peak carries its molecular mass and, with tandem mass spectrometry, a fragmentation pattern giving the class and often the exact identity of the constituent, matched against standards and libraries; a peak co eluting with a marker under ultraviolet detection is confirmed or exposed by its mass. Second, profiling of the unknown: an extract's constituents are listed by mass and fragmentation in one run, which is how the composition behind a fingerprint is mapped and how adulterants, synthetic drug spikes and degradation products are found. Third, sensitive quantification by selected ion monitoring, where a marker is present at very low level or in a crowded matrix. The cautions belong with the power: mass alone does not distinguish isomers; ionisation varies between compounds, so peak size is not a measure of amount without calibration; and cost and expertise place LC-MS as the investigation tool behind the routine fingerprint, the instrument that explains the pattern HPTLC or HPLC records.

4.4 GC-MS in the characterisation of herbal extracts

Gas chromatography with mass spectrometry is the corresponding tool for the volatile fraction: volatile oils, monoterpenes and sesquiterpenes, and any constituent that can be made volatile, by derivatisation where needed. The gas chromatograph separates the vapourised mixture on a capillary column with a resolving power liquid methods cannot match for volatiles, and the mass spectrometer identifies each peak by its electron impact fragmentation pattern, matched against standard libraries, with the retention index as the second identifying coordinate. A volatile oil fingerprint by GC-MS is therefore a named list, not merely a pattern: a mint oil is characterised by its menthol and menthone peaks, a citrus oil by its limonene led profile, and adulteration shows as peaks and ratios the genuine profile does not contain. The syllabus pairing of LC-MS and GC-MS is a coverage statement: LC-MS characterises the non volatile majority, glycosides, saponins, alkaloids and phenolics, GC-MS the volatile minority, and a full characterisation uses both.

4.5 Structure elucidation of phytoconstituents: the general method

This unit ends where Unit 5 begins, with structure elucidation stated as a general method. To elucidate a structure is to prove the complete constitution of a pure constituent: its molecular formula, its skeleton, the position of every functional group and substituent, and where the method allows, its stereochemistry. The evidence is gathered in a fixed order, each step narrowing what the molecule can be. The molecular formula comes from mass spectrometry, checked against the carbon count of the 13C NMR spectrum. The degrees of unsaturation from the formula state how many rings and multiple bonds the structure must contain. The ultraviolet spectrum reports the conjugated system, if any, and shift reagents in flavonoid work report the hydroxylation pattern. The infrared spectrum reports the functional groups, each in its standard region. The 1H NMR spectrum reports the hydrogen environments: how many kinds, in what numbers by

integration, in what surroundings by chemical shift, and next to what by splitting. The 13C NMR spectrum reports every carbon environment, with editing experiments distinguishing CH3, CH2, CH and quaternary carbons. Two dimensional NMR then assembles the fragments into one skeleton, and the assembled structure is checked against every spectrum, since a correct structure must explain all of the evidence, not most of it. Unit 5 works this method on the nine named compounds.

4.6 Unit 4 in one view

A herbal extract is characterised as a whole by its fingerprint, compared entire against an authenticated reference for identity, consistency and adulteration. HPTLC delivers the routine fingerprint and marker assay on one plate. LC-MS identifies and profiles the non volatile constituents by mass and fragmentation. GC-MS names the volatile constituents peak by peak. And structure elucidation proves a pure constituent's structure by the ordered agreement of mass, ultraviolet, infrared and NMR evidence, the method Unit 5 now applies.

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